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THE AMMONIACAL NITROGEN OF PEATS AND HUMUS SOILS.

PART I.

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THE detailed examination of humus soils of various kinds is becoming of increasing importance in view of the schemes now on foot for the reclamation of much heath and moor land. One of the most important things to establish has seemed to the authors to be the amount of nitrogen existing in the form of ammonia or of ammonium compounds, as it is possible that a knowledge of this figure might form a basis for a more scientific classification of soil organic matter than at present exists.

In those humus soils where so-called acid conditions obtain it might be expected that the amount of ammoniacal nitrogen would be high, as in these cases it would have the maximum opportunity for accumulation. Consequently the preliminary work was carried out with soils of this kind; mostly so-called acid peats.

It had previously been noticed by one of us that the method adopted by Russell[1] for the determination of ammonia in arable soils seemed to give concordant results when applied to humus soils. The method consists essentially in the distillation of an adequate amount of the peat with magnesia at a temperature of $40°$ under as low a pressure as can be obtained with an ordinary water pump.

It was decided to employ magnesia as the base for the liberation of ammonia, because the manipulation is easier, and because from Russell's results and from the results of our preliminary experiments there seemed no reason to doubt but that the magnesia was attacking a definite group of substances in the peat.

[1] Morison and Sothers, This *Journal*, vol. 6, pt. I, p. 88.

The actual details of the experiment are as follows.

The apparatus consisted of a round-bottomed litre flask fitted with a trap and another tube, the latter passing down to the bottom of the flask. This tube after leaving the flask was bent twice at right angles and tapered off into a fine capillary. The capillary was enclosed in a small bottle through the cork of which passed a small open tube. The bottle contained sulphuric acid, above the surface of which the capillary terminated. This device permitted of a slow stream of ammonia-free air passing into the flask during the distillation, in order to avoid bumping.

The trap in the distilling flask was connected by means of a glass tap to a 100 c.c. pipette which passed through a cork fitting into a filter flask containing sulphuric acid, into which the end of the pipette dipped.

The side-tube from the filter flask was connected to another glass tap, which was in turn connected to a water pump. Between the tap and the pump a manometer and safety bottle were inserted

The glass taps were found necessary when it was decided to conduct three distillations in parallel, the three distilling flasks in the same water bath being connected to the same capillary, and the same water pump being used to evacuate the whole apparatus.

At the end of the distillation the glass taps on either side of each filter flask were turned off, and the pipette and flask disconnected for purposes of titration.

The preliminary determinations were made with air-dried peats, 30 grs. of which were found to be a convenient weight for purposes of distillation.

100 c.c. of N/100 sulphuric acid were used in the filter flasks and, after the distillation, the excess of acid was determined by titration with N/100 potassium hydroxide, using methyl orange as indicator.

The method differs from that of Russell in that the distillation is only continued for three hours, and the liquid is not all distilled, a procedure which did not seem advisable in the case of a substance containing so much nitrogenous material as these soils.

In Table I are given the results obtained from the distillation of peat in the manner described for five hours, fresh acid being used every hour for the absorption of the ammonia given off.

The results are expressed as percentage of nitrogen in air-dried peat.

In every case a very marked drop occurs after the second hour, and after the third hour a small amount comes over which remains practically constant during the fourth and fifth hours.

<div align="center">TABLE I.</div>

	A	B	C
1st hour	·00323	·00390	·00508
2nd „	·00508	·00551	·00529
3rd „	·00230	·00230	·00207
4th „	·00207	·00184	·00184
5th „	·00207	·00162	·00184

The ammonia which is most easily removable comes over in the first two hours. Distillations for three hours would make certain of the removal of the whole of the nitrogen arising from the decomposition of the more unstable compounds present, while as little as possible of the nitrogen from more stable compounds would be included in this result.

The nitrogen should come from ammonia present either as ammonium compounds and absorbed ammonia, or from organic compounds which are hydrolysed with comparative ease.

The amount of magnesia taken was fixed at three grams as a result of a set of determinations of which Table II contains examples.

These determinations were made by distilling the peat for three hours with 1 gram of magnesia, then adding a second gram and continuing the distillation for another three hours, and similarly for a third gram.

<div align="center">TABLE II.</div>

	Nitrogen per cent.		
	Soil (1)	Soil (2)	Soil (3)
1st gr. MgO	·005866	·007863	·006878
2nd „	·001471	·000475	·000984
3rd „	·000992	·000345	·000051

Determinations were then made of the amounts of ammonia set free in this way from a series of humus soils.

The origin of the soils and the results are given in Table III.

It will be seen from these results that large quantities of ammonia may be set free from humus soils, which quantities are very much larger than the amounts set free from ordinary soils when treated in a similar way. To demonstrate this determinations were made in three ordinary soils:

N as NH₃, per cent.

(1) A coarse sand in rough permanent grass.   { ·00065 / ·00060 }

(2) Arable soil on limestone gravel subsoil.   { ·00027 / ·00037 }

(3) Arable alluvial soil on gravel subsoil.   { ·00046 / ·00046 }

TABLE III.

Origin and Description	Reaction	Ammoniacal Nitrogen evolved expressed as percentage of air-dried peat	
Irish Peats :			
Shaughnessy's Bog. Old deposit of peat lying under permanent grass of fair quality. Carboniferous limestone. Altitude, 200 ft. S.W. Co. Limerick	Neutral	·0079 ·0075	D
The Abbey Bog. Very shallow small bog on carboniferous limestone in S. W. Co. Limerick	Neutral	trace ·0011	E
Knockballyboy, King's Co. Carboniferous limestone. Surface peat	Neutral	·0053 ·0051	F
Shanagolden, S W. Co. Limerick. Millstone grit. Altitude, 600–700 ft. Surface covered with heather, bog cotton and osmunda	Acid	·0227 ·0214	A
Red bog. Surface, 0″–9″ (see above)	Acid	·0129 ·0118	B
Red bog. Surface, 9″–18″	Acid	·0135 ·0121	C
Meeneen, Portumna, Co. Galway. Surface peat...	Acid	·0235 ·0224	G
Scotch Peat :			
Corrour, Inverness. Surface peat. Altitude, 1800 ft.	Acid	·0296 ·0261 ·0260 ·0285	H

The amount of ammonia obtained from neutral peats is very much less than that obtained from so called acid peats.

The large quantities of nitrogen obtained in some peats can come from two sources, either from organic compounds capable of hydrolysis by the magnesia, or from ammonia or ammonium salts absorbed by the peat.

It was considered that a qualitative distinction might be made between the two by determining the amount of ammonia which could be removed by extraction with water.   The removal of any considerable quantity by water would show that much of it was present adsorbed by the peat.

A suitable quantity of air-dried peat was shaken continuously for 24 hours with ammonia free distilled water and filtered, and an aliquot part distilled with magnesia as before.

Results of this operation are given in Table IV. In the first column are given amounts of ammoniacal nitrogen expressed as percentages of air-dried peat; in the second column these amounts are expressed as percentages of the amount of ammoniacal nitrogen removed by direct distillation with magnesia:

TABLE IV.

Soil		Reaction	I	II
Shanagolden	... ...	Acid	·0141 ·0139	63·4
Red Bog 9″-18″	... ...	Acid	·0068 ·0059	50·2
Corrour	... ...	Acid	·0215 ·0215 ·0225	79·7
Meeneen, surface	... ...	Acid	·0119 ·0123	52·4
,, 3 ft depth	...	Acid	·0071 ·0079	63·1
,, 6 ft. depth	...	Acid	·0109 ·0112	69·5

These results are important as showing that the soil water in peat contains ammonia or ammonium compounds which are in equilibrium under the conditions of the experiment with the same substances adsorbed by the colloidal bodies in the humus. Further work is necessary before it is possible to differentiate quantitatively between the nitrogen obtained from adsorbed ammonium compounds and that arising from the decomposition of organic bodies. In Table IV results have been given with peats obtained from 3 feet and 6 feet from the surface as well as from the surface itself, and the figures show that the amounts of ammonia capable of extraction by water differ considerably at the different depths.

This has been found to be a very general phenomenon both in the case of these water extractions and in the case of direct distillations with magnesia. Examples of the latter are given in Table V, which shows the quantities of ammoniacal nitrogen removed by ordinary distillations with magnesia, expressed as percentages of air-dried peats The results are of 3 soils, and to 3 depths of soil.

The variations observed are great and, as was shown in Table IV, also occur with the amounts of ammonia extracted by water. So far it has been impossible to trace any definite relation between the two sets of figures.

TABLE V.

		Corrour		Meenoen	Killeen
Surface	...	·0296		·0235	·0102
		·0261		·0224	·0107
		·0261			
		·0285			
3 ft.	... ...	·0184	·0161	·0101	·0120
		·0189	·0167	·0104	·0111
		·0186	·0195		
			·0171		
			·0195		
6 ft.	... ...	·0097		·0148	·0173
		·0075		·0170	
		·0080			
		·0075			

The results of these preliminary investigations may be summarised as follows:

1. Distillation with magnesia under the conditions already described removes an amount of ammonia which is fairly constant for the same peat. This amount is many times greater than in the case of arable soils.

2. Much of the ammonia can be removed by water alone, and no constant ratio has been found between this amount and that obtained by direct distillation.

3. The amounts of ammonia removable by direct distillation with magnesia and by solution in water vary considerably with the depth from which the sample has been obtained.

The nature and source of this ammoniacal nitrogen and the conditions of its solution in the soil water are now being investigated.

THE ESTIMATION OF CARBOHYDRATES. V.

THE SUPPOSED PRECIPITATION OF REDUCING SUGARS BY BASIC LEAD ACETATE.

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GILL[1] in 1871 first pointed out that when an excess of basic lead acetate is added to a solution of invert sugar the negative rotation of the latter is greatly reduced owing to the formation of a *soluble* lead compound of laevulose. If sufficient lead solution is added the negative rotation may become a positive one; thus in one experiment quoted by Gill a negative reading of -28^c was transformed into a positive value of $+57°$. The change of rotation was not, however, permanent and on removing the lead or on acidifying the solution the original rotatory power was restored. The change of rotation was attributed to an effect of the lead on the laevulose only; a solution of dextrose was practically unaffected by the presence of basic lead acetate. Since Gill's paper the effect of basic lead acetate as a source of error in sugar analysis has been the subject of numerous papers especially by Pellet[2], Svoboda[3], Edson[4], Prinsen Geerligs[5], Watts and Tempany[6] and Eynon[7].

As a result of this work the belief has grown up that when laevulose is present in a sugar solution which is defecated by basic lead acetate, a portion, if not the whole, of the laevulose is thrown down in the

[1] *Trans. Chem. Soc.* 1871, **24**, 91

[2] *Bull. Assoc. Chim. Sucr.* 1891, **9**, 439; 1896, **14**, 28 and 131; 1897, **15**, 605; 1899, **16**, 1007 and 1147; 1900, **17**, 52; 1904, **22**, 744; 1913, **31**, 205; 1914, **32**, 909. *J. Fabr. Sucr.* 1899, **40**, No. 15. *Sucrerie Indigène*, 1904, **64**, 67.

[3] *Zeit. Ver. Rubenzuckerind.* 1896, **46**, 107.

[4] *Bull. Assoc. Chim. Sucr.* 1890, **8**, 323; 1891, **9**, 552.

[5] *Archiv Zuckerind. Javas*, **6**, 914; *Zeit. Ver. Deut. Zuckerind.* 1908, 932; *Int. Sugar J.* 1908, **10**, 432.

[6] *J. Soc. Chem. Ind.* 1908, **27**, 53.

[7] *7th Int. Congress App. Chem.* 1909, **5**, 193.

lead precipitate, thus causing considerable error in the analysis. 'Pellet, has stated that in some cases as much as 23 % of the laevulose is precipitated at the ordinary temperature, whilst at 50° the whole of the laevulose is removed. The view that laevulose is actually *precipitated* has been universally adopted in the standard treatises on sugar analysis[1].

It is true that Watts and Tempany, like Gill himself, showed that if to a solution of pure invert sugar basic lead acetate is added, although the rotation is greatly changed in the positive direction no laevulose is actually precipitated and on adding just sufficient acetic acid to combine with the lead oxide of the basic lead acetate present the original rotatory power is restored. Prinsen Geerligs, however, maintained that whilst this is true of pure solutions of invert sugar or laevulose it is not so when other substances such as sodium chloride or sulphate are present or in fact any compound capable of forming a precipitate with the lead. In such cases Geerligs considered that the laevulose was actually precipitated and lost; whether it was regarded as thrown out *per se*, in an insoluble form or co-precipitated by a process of adsorption is not made clear. In adopting this view the early statement of Gill in 1871, that when the lead is precipitated by sulphur dioxide the original rotation of the invert sugar is restored, was entirely ignored.

The object of the present paper is to show that whilst the experimental facts recorded by the writers cited above are perfectly correct they have been misinterpreted. The results given below show that, at least in dilute solutions, laevulose is never *precipitated* by basic lead acetate even in presence of salts such as chlorides, sulphates or carbonates. *No loss of laevulose occurs, indeed, unless the excess of basic lead acetate is allowed to act for some length of time upon the sugar before the lead is precipitated.* If to the solution of pure laevulose basic lead acetate is added in excess (5 c.c. or 10 c.c. to 50 c.c. of solution) and the lead is *immediately* precipitated by sodium carbonate or sodium sulphate, practically 100 % of the laevulose is recovered in the solution. But if, on the other hand, the basic lead acetate is left with the sugar solution for different lengths of time, for example, 15 minutes, 1 hour, 24 hours, and the lead is then precipitated by the same reagents, increasing proportions of laevulose are found to have disappeared. The amount of laevulose lost depends solely on the length of time the

[1] For example by von Lippmann in Lunge's *Chem Techn. Untersuch. Methoden,* 3, 485 (5th Ed.): by Ling (Thorpe's *Dict. App. Chem* article "Sugar," p. 244). Compare also Allen's *Commercial Organic Analysis,* 4th Ed., 1, 311.

basic lead acetate is allowed to act upon it. As the time increases the solution becomes more and more yellow in colour but in no case does a precipitation of laevulose become visible. The data given on pages 12–15 show that the sugar remaining in solution no longer consists solely of laevulose. Not only does there appear to be a deficiency of 20 to 30 % of laevulose but values obtained for this sugar by reduction are always far higher—13 to 14 %—than the values calculated from the rotatory power. This points to a substance being formed from the laevulose which has a considerably lower reducing power than this sugar and at the same time differing from it even more in rotatory power. Such a substance is the so-called *glutose*, $C_6H_{12}O_6$, obtained by Lobry de Bruyn and van Ekenstein[1] on heating a 20 % solution of laevulose with aqueous lead hydroxide at 70–100°; it is described as being nearly optically inactive and as having a reducing power about one-half that of dextrose. It was found to be present in small quantities in certain kinds of colonial molasses, being formed probably by the action of alkali (lime) on the invert sugar during the processes of evaporation in the factory[2].

The data given in the experimental part of this paper point to this substance being formed *at the ordinary temperature* by the action of basic lead acetate on dilute solutions of laevulose. The basic lead acetate acts in fact in the same way as lead hydroxide itself. The higher the temperature the more rapid is the disappearance of the laevulose, so that heating a solution containing reducing sugars and basic lead acetate should always be avoided.

It is well known that Pellet advocates the use of normal lead acetate, as suggested by Edson, in place of the basic salt, in clarifying liquors which contain reducing sugars, so as to avoid the supposed precipitation of laevulose. In most cases, however, the normal acetate is far less effective as a clarifying agent and it frequently leaves in solution optically active substances, such as gums, which are completely eliminated by the basic acetate and thus prevented from interfering with the analysis. The writer's experience with many different kinds of leaf material, rich in reducing sugars, would show that if the basic lead acetate is added carefully in small quantities at a time until the precipitation of the impurities is *just* complete[3] and the actual excess

[1] *Rec. Trav. Chim.* 1897, **16**, 262.

[2] Pellet, *Bull. Assoc. Chim.* 1899, **16**, 1181 and 1902, **19**, 834.

[3] It is usually quite easy by testing small portions of the filtrate to hit off the point at which this occurs to within 1 or 2 c.c., even when relatively large quantities (for example, 200 to 300 c.c.) of the basic lead solution have to be used.

of basic lead acetate solution is not allowed to exceed about 5 c.c. in 300 to 500 c.c. of the solution, *there is no loss whatever of laevulose or other reducing sugars.* Care must always be taken that the excess of basic lead acetate is not left for any length of time with the sugar solution; the latter after precipitation should be immediately filtered on a Buchner funnel, and after thoroughly washing, the excess of lead should be precipitated as soon as possible with sodium carbonate or sodium sulphate. The solution can then be diluted to a known volume, when it remains perfectly stable without any change occurring in the sugars present[1].

Experiment has shown that a mixture of saccharose, reducing sugars and tannin can be precipitated in this way without any loss of sugar occurring if care be taken to *avoid using any considerable excess of basic lead acetate during the precipitation.* Parkin[2] has given an example of this kind in which the following results were obtained in a test analysis:

	Tannin added and precipitated with 5 c.c. of basic lead acetate	Control in distilled water
Saccharose	0·487 grm.	0·489 grm.
Dextrose	0·204 ,,	0·201 ,,
Laevulose	0·244 ,,	0·244 ,,
Total sugar	0·935 ,,	0·934 ,,

In this case tannin was added to the solution in amount just sufficient to be carried down by the 5 c.c. of basic lead acetate; the slight excess of lead present was not removed and it is seen not to have influenced the result.

In agreement with the view the writer puts forward that no loss of laevulose ever occurs by actual precipitation, but that when such appears to take place it is caused by a transformation of the sugar by the alkaline lead solution, are the facts recently recorded by Le Docte[3]. The latter showed that whereas in the hot aqueous digestion process of extracting sugars from sugar beet in presence of basic lead acetate, the polarisation due to laevulose (which was purposely added) *disappeared* entirely, it was not changed at all or only very slightly when

[1] If the solution is to be kept for any time a little toluene should be added and the mixture well shaken. If sodium carbonate is used any large excess should be avoided, tests being made during the precipitation until exactly the right quantity has been added. When these precautions are observed the solution can be kept for months without any change, even inversion of saccharose, taking place.

[2] *Biochem. J.* 1911, 6, 12.   [3] *Sucrerie Belge*, 1912, 275.

the digestion was effected in the cold. Similarly, when the actual hot digestion was carried out in the absence of basic lead acetate and the latter was then added *after cooling*, the polarisation due to laevulose (or dextrose) was not in the least interfered with. Le Docte concluded that, as stated above, basic lead acetate should never be added to the hot solution of sugars.

Laevulose is far more sensitive to the action of basic lead acetate in the cold than dextrose, just as it is far more easily decomposed, for example, by acids[1]. The writer has found that dextrose and maltose remain practically unchanged in presence of a considerable excess of basic lead acetate and Le Docte (*loc. cit.*) also states that in presence of dextrose only, the hot digestion with basic lead acetate can be carried out without loss of reducing sugar. The fact that no loss of either dextrose or maltose occurs in presence of basic lead acetate greatly simplifies the analysis of solutions containing these substances. The writer[2] has shown, for example, that basic lead acetate can be used in removing impurities in the estimation of starch by means of taka-diastase, without any loss whatever occurring of either of these sugars.

In sugar analysis any large excess of basic lead acetate should always be avoided and in most cases should be removed before taking the actual polarisation readings, either by means of sulphurous acid, as recommended by Pellet, or by sodium carbonate or sulphate. If an excess of basic lead acetate is left it combines with the reducing sugars (dextrose, laevulose and maltose) forming *soluble* lead compounds which have an entirely different rotation from the sugars themselves. This is particularly the case with laevulose (or invert sugar) the negative rotation of which may become positive in presence of excess of basic lead acetate. In working with cane juices the experiments of Watts and Tempany (*loc. cit.*) show that such slight excesses of basic lead acetate as are ordinarily used in practice do not sensibly interfere with the results.

[1] Davis and Daish, This *Journal*, 1914, **5**, 454.

[2] This *Journal*, 1914, **6**, 152.

EXPERIMENTAL.

A. *Laevulose experiments.*

SERIES I. *Solution* 1. An approximately 1 % solution of Kahlbaum's pure laevulose from inulin was prepared. Under Brown, Morris and Millar's conditions[1]

$\qquad$ 20 c.c. gave (1) 0·4528 grm. CuO

$\qquad\qquad\qquad$ (2) 0·4521 $\quad$,,

$\qquad$ Average = 0·4525 grm. CuO = 0·2047 grm. laevulose.

The concentration of the laevulose solution is therefore 1·0235 grms. per 100 c.c. In a 400 mm. tube the rotation observed was − 3·663° at 20° (sodium flame).

Expt. 1. *Basic lead acetate precipitated with sodium carbonate immediately after being added to the laevulose solution.*

50 c.c. of solution 1 (− 0·5117 grm. laevulose) were mixed with 5 c.c. of basic lead acetate (sp. gr. 1·25, prepared according to Allen's *Commercial Organic Analysis*, i. 308); powdered sodium carbonate was then added, little by little, until the whole of the lead was just precipitated. A very slight excess of the carbonate was used, so that the solution was just faintly alkaline. The solution was diluted to 100 c.c. at 15° and the rotatory and reducing powers were determined. The values given are the mean of two or more separate experiments.

TABLE I.

	Reduction			Polarisation	
CuO from 25 c.c. grms.	Laevulose calculated per 100 c.c. soln. 1 grms.	% of laevulose remaining	α_p^{20} in 400 mm. tube	Laevulose remaining calculated from rotation %
0·2930	1·016	99·3	− 1·840°	100·5

Thus practically 100 % of the laevulose present is found after the treatment with basic lead acetate and sodium carbonate. The reduction result is a trifle low and the polarisation value a trifle high, but the differences are within the limits of experimental error; the average of the two methods is 99·9 %.

Expt. 2. *Basic lead acetate precipitated immediately by sodium sulphate.*

5 grms. of sodium sulphate ($Na_2SO_4 . 10H_2O$) were dissolved in 50 c.c. of solution 1 (= 0·5117 grm. laevulose) and 5 c.c. of basic lead

[1] *Trans. Chem. Soc.* 1897, **71**, 72.

acetate solution added and the mixture well shaken; within 10 minutes a small quantity of sodium carbonate was added to complete the precipitation of the lead and the solution diluted to 100 c.c. at 15°.

TABLE II.

	Reduction			Polarisation	
CuO from 25 c.c. grms.	Laevulose calculated per 100 c.c. soln. 1 grms.	% of laevulose remaining	a_{0}^{20} in 400 mm. tube		Laevulose remaining calculated from rotation %
0·2898	1·004	98·2	− 1·847°		100·9

The results given show an average of 99·6 %. The reduction value is again slightly low and the polarisation slightly high, probably owing to a slight influence of the salts present.

Expt. 3. *Laevulose left for different times with basic lead acetate.*

50 c.c. of laevulose solution 1 were mixed with 5 c.c. (or 10 c.c.) of basic lead acetate solution and left for different times in a stoppered flask. The lead was then precipitated with sodium carbonate or sulphate and the solutions made up to 100 c.c. at 15°. In each case the average of two or more concordant values is given.

TABLE III.

		Reduction values			Polarisation values		
Time left	c.c. of basic lead acetate added	grms. CuO from 25 c.c.	Laevulose found calc. per 100 c.c. solution 1 grms.	% of laevulose remaining	a_{0}^{20} in 400 mm. tube	Laevulose remaining %	Remarks
23 hours	5	0·2400	0·8235	80·5	− 1·231°	67·2	Solution very pale yellow
23 „	10	0·1960	0·6681	65·3	− 0·922°	50·35	„ darker yellow
40 „	5	0·2045	0·6971	68·1	− 1·009°	55·1	„ „
72 „	5	0·1877	0·6386	62·4	− 0·885°	48·3	„ „

These experiments show clearly that the effect of leaving the basic lead acetate with the laevulose is to cause part of the latter sugar apparently to disappear; as the time increases, more and more laevulose is apparently lost. The greater the amount of basic lead acetate added the greater is the effect in the same time (compare the experiments with 5 c.c. and 10 c.c. respectively of lead acetate). As the laevulose is transformed into *glutose*, a sugar with almost a negligible rotatory power and a reducing power about half that of laevulose, the amount of sugar *remaining, calculated as laevulose*, is always considerably lower by polarisation than by the reduction method.

SERIES II. A fresh approximately 1 % solution of laevulose was used. (Solution 2.)

25 c.c. gave (1) 0·2960 grm., (2) 0·2960 grm., (3) 0·2966 grm. Average = 0·2962 grm. CuO.

Hence concentration of laevulose = 1·028 grms. per 100 c.c.

Rotation observed in 400 mm. tube $a_D^{20°} = - 3·808°$

Specific rotation of the laevulose $[a]_D^{20°} = - 92·7°$.

50 c.c. of solution 2 were mixed with 5 c.c. of basic lead acetate and the mixture left 24 hours. Sodium carbonate was then added and the solution diluted to 100 c.c.

TABLE IV

			Reduction values		Polarisation values	
Time	c.c. of basic lead acetate	CuO from 25 c.c. grms.	Laevulose calc on 100 c c of original solution	% of laevulose remaining	a_D in 400 mm. tube	% of laevulose remaining
24 hours	5	0 2580	0 8820	86 5	- 1 496°	78·6

SERIES III. A fresh solution of laevulose was used (solution 3).

25 c.c. gave 0·3164 grm. CuO (average of three values).

Hence laevulose = 1·1025 grms in 100 c.c.

Rotation observed in 400 mm. tube 1·060°.

$$[a]_D^{20} \quad 92·0°.$$

50 c.c. of solution 3 were mixed with 5 c c. of basic lead acetate. After 6 days the solution had become decidedly yellow It was precipitated with sodium carbonate and treated as shown in column 3

TABLE V.

			Reduction values			Polarisation values	
Time	Basic lead acetate	Conditions	CuO from 25 c c grms	Laevulose calc on 100 c c of solution 3	% of laevulose remaining	a_D in 400 mm. tube	% of laevulose remaining
6 days	5 c.c.	After 6 days made acid with acetic acid and diluted to 100 c c. *without removing the lead*	0 2548	0 8768	79 5	- 1 159°	57 1
6 days	5 c.c.	Acidified with acetic acid and lead precipitated by adding sodium sulphate	0 2464	0 8458	76 7	- 1·210°	59·6

As in the earlier experiments, there is the same wide difference for apparent laevulose remaining by the reduction and polarisation methods. As the time is considerably greater (6 days) so that the amount of

glutose formed has increased, the divergence is far greater between the two sets of values, than in the earlier experiments (23 to 72 hours, Table III).

B. *Experiments with Invert Sugar.*

SERIES IV. The following set of experiments was made by Dr H. Limbosch.

A 1 % solution of saccharose (solution A) was prepared by dissolving 5·000 grms. of the pure sugar in 500 c.c. of water.

Rotation in 400 mm. tube, $a_D^{20} = +2·686°$.

100 c.c. of solution A were inverted by heating 10 minutes with 10 grms. of citric acid. The solution was exactly neutralised by sodium hydroxide, and diluted to 200 c.c. $=$ solution B.

TABLE VI.

			Reduction values			Polarisation values	
Experi-ment	Treatment	Time left	c.c. of basic lead	CuO from 50 c.c. solution C grms.	% of invert sugar remaining	a_D of C in 400 mm. tube	% of invert sugar remaining*
1	50 c.c. of B mixed with 5 c.c. of basic lead solution and immediately precipitated with solid sodium carbonate. Made to 100 c.c. —solution C	0 mins.	5	0·3151	99·0	−0·203°	100·3
2	50 c.c. of B left 10 mins. with 5 c.c. basic lead acetate. Then as in 1	10 ,,	5	0·3135	99·3	−0·201°	100·0
3	50 c.c of B left 60 mins. with 5 c.c. of basic lead acetate. Then as in 1	60 ,,	5	0·3086	97·7	− 0·195°	99·4
4	50 c.c. of B left 24 hours with 5 c.c. of basic lead acetate. Then as in 1	24 hrs.	5	—	—	− 0·141°	93·2

* The polarisation calculations were made by assuming that the *change* of rotation of a 1 % solution of saccharose on inversion is 3·488° in a 400 mm. tube at 20°.

The values given in Table VI show that even when the basic lead acetate is left 10 minutes in contact with the invert sugar solution and is then precipitated by sodium carbonate the loss of laevulose is less than 1 % of the sugar present. After 60 minutes, however, the loss of sugar becomes quite appreciable, whilst after 24 hours the loss is considerable. It is noteworthy that in the above experiments the polarisation values are slightly higher than the reduction figures:

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NOTES ON THE NATURE OF THE PHOSPHATES CONTAINED IN MINERAL PHOSPHATES.

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THE present work has been undertaken with the object of ascertaining the actual compounds of phosphoric acid and lime present in rock phosphates, a better knowledge of these compounds being of some agricultural importance.

The following table shows the analysis of the rock phosphates used.

	Makatea Island	Florida Pebble	Algerian	Gafsa	Tunisian	Belgian	Apatite
Calcium oxide	52·38	47·10	46·13	43·30	48·40	50·55	55·45
Phosphoric acid	38·24	31·50	27·27	25·35	26·13	19·80	39·49
Carbon dioxide	1·69	3·04	6·70	5·50	9·03	17·22	2·23
Moisture	1·46	1·00	0·72	3·26	0·98	} 3·60	—
Combined moisture and organic matter	3·39	2·41	3·28	4·39	3·21		
Ferric and alum. oxide	1·01	1·60	2·59	4·98	4·25	1·09	—
Magnesium oxide	1·35	1·90	2·02	1·72	0·90	1·14	—
Sand, etc.	0·28	7·03	8·12	7·56	4·65	4·28	0·38
Undetermined	0·20	4·42	3·17	3·94	2·45	2·32	2·45
	100·00	100·00	100·00	100·00	100·00	100·00	100·00

Each of the above phosphates was ground, and 5 grams submitted to consecutive half-hour extractions with 500 c.c. of 2 per cent. citric acid. The harder phosphates, such as Florida pebble phosphate and Makatea Island phosphate, were separated into various grades of fineness by means of the 1 mm. sieve, and sieves with 30, 60, 90 and 100 holes to the linear inch. Five grams of each of the five portions so obtained from both phosphates were submitted to five consecutive extractions with 2 per cent. citric acid. By this means a large number of citric acid extracts of these two rock phosphates were obtained.

Five grams of each of the phosphates were calcined in a Fletcher muffle furnace in order to determine what, if any, changes were produced by heating.

Makatea Island Phosphate.

The results with Makatea Island phosphate are given in the following tables.

	Refuses "1 mm. sieve"			Refuses "30" sieve		
	% P_2O_5	% CaO	Ratio P_2O_5 : CaO	% P_2O_5	% CaO	Ratio P_2O_5 : CaO
1st extract	3·93	5·40	1 : 1·37	4·98	7·00	1 : 1·40
2nd „	3·66	5·10	1 : 1·39	4·85	6·70	1 : 1·38
3rd „	3·47	4·80	1 · 1·38	4·56	6·40	1 : 1·40
4th „	3·28	4·55	1 : 1·39	3·94	5·33	1 : 1·39
5th .	2·91	4·10	1 : 1·41	3·70	5·20	1 : 1·40
Extract totals	17·25	23·95	1 : 1·388	21·93	30·63	1 : 1·39
Actual totals	38·90	53·38	1 : 1·372	39·24	53·75	1 : 1·37

	Refuses "60" sieve			Refuses "90" sieve		
	% P_2O_5	% CaO	Ratio P_2O_5 : CaO	% P_2O_5	% CaO	Ratio P_2O_5 : CaO
1st extract	6·40	9·00	1 : 1·40	7·47	10·65	1 : 1·42
2nd „	6·06	8·58	1 : 1·41	7·07	9·90	1 : 1·39
3rd „	5·44	7·52	1 : 1·38	5·95	8·40	1 : 1·41
4th „	4·77	6·72	1 : 1·40	5·46	7·50	1 : 1·37
5th „	4·30	6·00	1 : 1·37	4·82	6·70	1 : 1·39
Extract totals	26·97	37·80	1 : 1·40	30·77	43·15	1 : 1·39
Actual totals	39·23	53·90	1 : 1·38	39·09	53·57	1 : 1·37

	Refuses "100" sieve			Calcined. Passes "100" sieve		
	% P_2O_5	% CaO	Ratio P_2O_5 : CaO	% P_2O_5	% CaO	Ratio P_2O_5 : CaO
1st extract	9·25	13·01	1 : 1·40	6·58	9·50	1 : 1·44
2nd „	8·26	11·46	1 : 1·38	6·32	8·58	1 : 1·36
3rd „	7·54	10·50	1 : 1·39	5·55	7·51	1 : 1·35
4th „	6·03	8·55	1 : 1·41	4·63	6·29	1 : 1·36
5th „	4·22	5·75	1 : 1·36	3·78	5·16	1 : 1·36
Extract totals	35·30	49·27	1 : 1·39	26·87	37·04	—
Actual totals	38·24	52·38	1 : 1·37	38·24	52·38	1 : 1·37

The result of thirty determinations shows an average relationship P_2O_5 : CaO of 1 : 1·38 for the phosphoric acid and lime dissolving out in the citric acid solutions, and in hydrochloric acid. This ratio is quite different from the relationship in tricalcium phosphate 1 : 1·18, or in apatite 1 : 1·31, and agrees with the ratio of phosphoric acid to lime in a phosphate with the formula $2Ca_3P_2O_8 . CaO$.

The curves representing the rate of extraction of phosphoric acid from each of the portions are shown in Fig. 1. They are of a fairly definite nature, and are certainly not what would be derived from a mixture of phosphates.

1.   Passes "100" sieve   2.   Refuses "90" sieve
3   Refuses "60" sieve.   4.   Refuses "30" sieve.
5.   Refuses "1 mm." sieve.
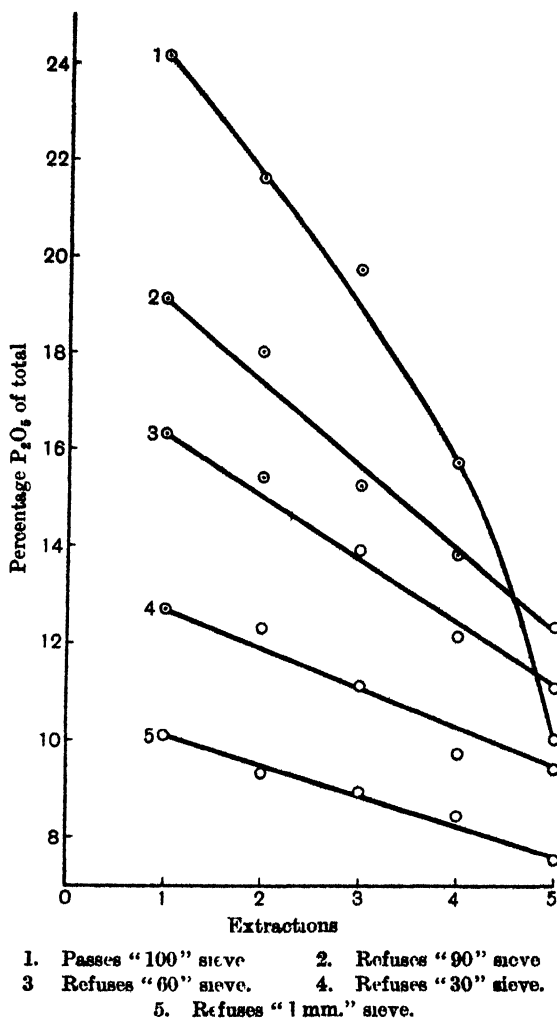
Fig. 1   Makatea Island Phosphate.

The solubility of the phosphate has been greatly decreased by calcining, and an examination of the relationship $P_2O_5 : CaO$ in the five extracts suggests that a change in the composition of the phosphate has been brought about by calcining.

Florida Pebble Phosphate.

Twenty half-hour extractions with 500 c.c. of 2 per cent. citric acid were made with various portions of this phosphate. The results obtained by extracting two portions are given below.

	Refuses "30" sieve			Passes "100" sieve		
	% P_2O_5	% CaO	Ratio $P_2O_5 : CaO$	% P_2O_5	% CaO	Ratio $P_2O_5 : CaO$
1st extract	4·00	5·98	1 1·49	6·18	9·23	1 1·49
2nd ,,	3·81	5·74	1 1·50	5·83	8·76	1 1·49
3rd ,,	3·63	5·38	1 1·48	5·46	8·24	1 1·50
4th ,,	3·23	4·90	1 1·51	5·14	7·72	1 1·50
5th ,.	3·00	4·63	1 1·54	4·31	6·36	1 1·47
Extract totals	17·67	26·63	1 : 1·50	26·92	40·31	1 : 1·49
Actual totals	33·27	49·13	1 : 1·47	31·50	47·10	1 : 1·49
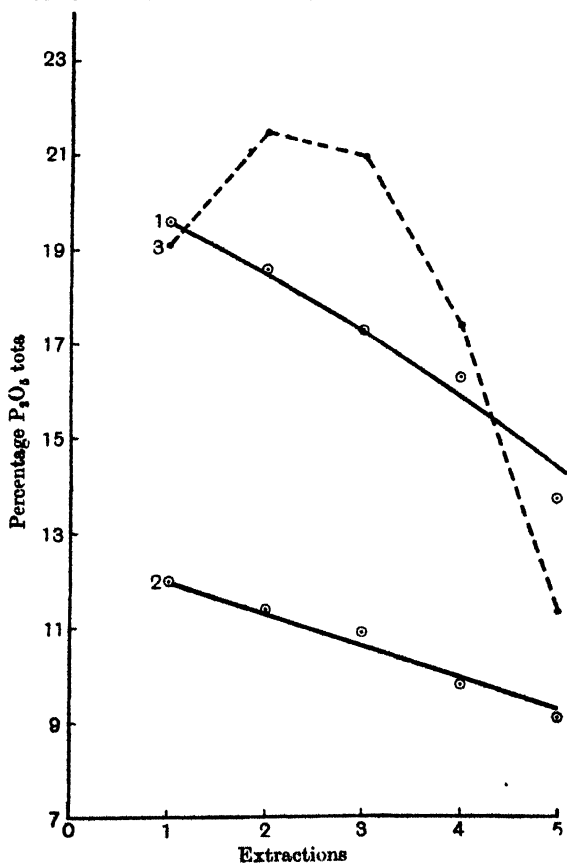
1. Passes "100" sieve.   2. Refuses ' 30" sieve.
3. Calcined phosphate.

Fig. 2. Florida Pebble Phosphate.

The solubility of Florida pebble phosphate in citric acid is noticeably less than that of Makatea Island phosphate. The average of twenty citric extracts gives a ratio P_2O_5 : CaO of 1 : 1·48. Extracts made with hydrochloric acid instead of citric acid give exactly the same ratios. The curves (Fig. 2) for the portion passing "100" sieve and the portion refusing "30" sieve are quite normal, and clearly demonstrate that whatever its nature, only one phosphate is being extracted.

A calculation based on the figures obtained for the citric acid extracts or hydrochloric extracts gives the formula $4Ca_3P_2O_8 . 3CaO$ (P_2O_5 : CaO :: 1 : 1·48) as representing the phosphate present in Florida pebble phosphate.

Calcined Florida Pebble Phosphate.

Five grams of the phosphate in a platinum dish were calcined for 4½ hours in a Fletcher muffle furnace. A sugar extract was made to remove any free lime, and this was followed by five half-hour extractions with 500 c.c. of 2 per cent. citric acid.

| | Calcined Phosphate | | Passes "100" sieve | |
	% P_2O_5	% CaO	% SiO_2	Ratio P_2O_5 : CaO
1st sugar extract	—	0·10	—	—
1st 2 % citric extract	6·01	9·56	1·27	1 : 1·59
2nd ,, ,,	6·77	10·00	—	1 : 1·48
3rd ,, ,,	6·63	9·07	—	1 : 1·35
4th ,, ,.	5·49	7·58	—	1 : 1·38
5th ,, ,,	3·88	5·44	—	1 : 1 40
Extract totals	28 78	41·75	1·27	—
Actual totals	31·50	47·10	—	1 : 1·49

That a change in the composition of the phosphates has been produced by calcining is obvious, and a glance at the curve on Fig. 2 shows that at least two phosphates are being dissolved by the citric acid. More phosphoric acid is soluble in the second citric extract than in the first. There is a big increase in the ratio P_2O_5 : CaO in the first citric extract, and as any free lime would be removed by the sugar extract it would appear that the phosphate dissolving in the first extract has a much higher lime content than the original phosphate. Silica derived from the sand in the sample of Florida pebble phosphate has also been brought into combination. The ratio P_2O_5 : CaO of 1 : 1·38 obtained in the 3rd, 4th and 5th extracts suggests at once the presence of the phosphate $2Ca_3P_2O_8 . CaO$ of which Makatea Island phosphate is composed. The formation of this phosphate would account

foʒ the increased total solubility of the calcined Florida pebble phosphate compared with the uncalcined, because, as has already been pointed out, Makatea Island phosphate is more soluble in citric acid than Florida pebble phosphate.

With regard to the phosphate in the first calcined citric extract, it is interesting to note that the phosphoric acid, calcium oxide and silica are dissolved in exactly the same proportion as they are present in the phosphate $(Ca_3P_2O_8)_2 \, 2CaO.SiO_2$.

Algerian Phosphate.

The ratios $P_2O_5 : CaO$ in the various citric acid extracts obtained from Algerian phosphates are given in the following table.

	Refuses "100" sieve			Passes "100" sieve		
	% P_2O_5	% CaO	Ratio $P_2O_5 : CaO$	% P_2O_5	% CaO	Ratio $P_2O_5 : CaO$
1st extract	5·87	10·76	: 1·82	6·65	12·92	1 : 1·94
2nd „	5·96	9·38	: 1·57	6·98	11·04	1 : 1·58
3rd „	5·00	7·80	] : 1·56	5·35	8·60	1 : 1·60
4th „	4·00	6·20	' : 1·55	4·00	6·30	1 : 1·57
5th „	2·80	4·48	: 1·60	2·47	3·94	1 : 1·59
Extract totals	23·63	38·62	: 1·57*	25·45	42·80	] : 1·58*
Actual totals	30·03	48·35	: 1·61	27·27	46·13	] : 1·69

* Excluding first extract.

If the first citric extracts in both portions are excluded a constant ratio of 1 : 1·58 is obtained for the phosphoric acid and lime dissolving in the eight citric extracts. The calcium oxide and phosphoric acid are dissolved out in the 2nd, 3rd, 4th and 5th extracts in exactly the same proportion as they are present in the phosphate $Ca_3P_2O_8 . CaO$.

Calcining produces a marked decrease in the solubility of Algerian phosphate in citric acid, and a still more marked alteration in the ratios in which the phosphoric acid and lime dissolve out in the various extracts.

Calcined Algerian Phosphate.

	% P_2O_5	% CaO	% SiO_2	Ratio $P_2O_5 : CaO$
1st sugar extract	—	1·18	—	—
2nd „ „	—	0·50	—	—
1st citric extract	3·33	8·62	2·13	1 : 2·59
2nd „ „	4·36	6·51	—	1 : 1·49
3rd „ „	3·78	5·32	—	1 : 1·40
4th „ „	3·10	4·18	—	1 : 1·35
5th „ „	2·29	3·24	—	1 : 1·40
Extract totals	16·86	29·55	2·13	—
Actual totals	27·27	46·13	—	—

All the free lime was removed by the two sugar extracts and the phosphate dissolving in the first citric extract is therefore one with a very high calcium oxide content and is also combined with silica. The' ratio P_2O_5 : CaO of 1 : 2·59 and the ratio SiO_2 : CaO of 1 : 4·05 are in agreement with the formula $(2Ca_3P_2O_8 . CaO) 3SiO_2 . 6CaO$. The second citric extract contains no silica and the ratio P_2O_5 : CaO is precisely the same as the ratio P_2O_5 : CaO in the second extract of calcined Florida pebble phosphate, and corresponds to the formula $4Ca_3P_2O_8 . 3CaO$, the phosphate present in uncalcined Florida pebble phosphate. The ratio P_2O_5 : CaO of 1 : 1·38 in the 3rd, 4th and 5th extracts corresponds with the phosphate $2Ca_3P_2O_8 . CaO$ which was found to be present in Makatea Island phosphate, and in the 3rd, 4th and 5th extracts of calcined Florida pebble phosphate.

Belgian and Gafsa Phosphates.

The results secured with Belgian and Gafsa phosphates are very similar to those recorded for Algerian phosphate. The ratio of phosphoric acid to lime is also 1 : 1·58, a ratio which corresponds to the formula $Ca_3P_2O_8 . CaO$.

With the calcined Belgian phosphate the following figures were obtained.

	% P_2O_5	% CaO	% SiO_2	Ratio P_2O_5 : CaO
1st sugar extract	—	17 32	—	—
2nd „ „	—	0·61	—	—
1st citric extract	3 57	7·80	1 16	1 : 2·18
2nd „ „	4·73	6 98	—	1 : 1·48
3rd „ „	4 28	6 20	—	1 : 1·44
4th „ „	3·16	4·53	—	1 : 1·43
5th „ „	2·14	3·08	—	1 : 1·44
Extract totals	17 88	46·52	1·16	—
Actual totals	19 80	50 55	—	—

The ratios in the 1st citric extract correspond to the phosphate $(Ca_3P_2O_8 . CaO)_4 6CaO . 3SiO_2$. The ratio in the 2nd extract again corresponds to the formula $4Ca_3P_2O_8 . 3CaO$, whilst the ratio in the 3rd, 4th and 5th extracts corresponds to the phosphate $3Ca_3P_2O_8 . 2CaO$, which also occurs in calcined Gafsa phosphate and calcined Tunisian phosphate.

The results obtained with calcined Gafsa phosphate are as follows.

	% P₂O₅	Passes "100" sieve % CaO	% SiO₂	Ratio P₂O₅ : CaO
1st sugar extract	—	0·62	—	—
2nd „ „	—	0·21	—	—
1st citric extract	5·20	12·30	3·26	1 : 2·36
2nd „ „	6 55	9 64	—	1 : 1·47
3rd „ „	5·77	8·40	—	1 : 1·46
4th „ „	4·49	6·46	—	1 : 1·43
5th „ „	2 29	3 30	—	1 : 1 44
Extract totals	24 30	40 93	—	—
Actual totals	25 35	43·30	—	—

The ratios in the 1st citric acid extract correspond to those in the phosphate $(Ca_3P_2O_8)_2\,6CaO\,.\,3SiO_2$, those in the 2nd and 3rd citric extracts to the phosphate $4Ca_3P_2O_8\,.\,3CaO$ (see Florida pebble phosphate), and those in the 4th and 5th extracts to the phosphate $3Ca_3P_2O_8\,.\,2CaO$ (see calcined Belgian phosphate).

Tunisian Phosphate.

The ratio P_2O_5 : CaO in the extractions secured from Tunisian phosphate are approximately 1 : 1·49. They therefore agree with the ratios in Florida pebble phosphate and are in accordance with the formula $4Ca_3P_2O_8\,.\,3CaO$.

Calcined Tunisian Phosphate.

	% P₂O₅	Passes "100" sieve % CaO	% SiO₂	Ratio P₂O₅ : CaO
1st sugar extract	—	3·07	—	—
2nd „ „	—	1 21	—	—
1st citric extract	4 46	11·70	2·94	1 : 2·62
2nd „ „	4 58	6·80	—	1 : 1·49
3rd „ „	3 54	5·10	—	1 : 1·44
4th „ „	2 56	3·60	—	1 : 1·40
5th „ „	2·07	2·86	—	1 : 1 38
Extract totals	17 21	34·34	2·94	—
Actual totals	2b 13	48·40	4·65	—

The ratios P_2O_5 : CaO and SiO_2 : CaO in the 1st citric extract closely resemble those in the 1st citric extract of calcined Algerian phosphate, and it would thus appear that the phosphate

$$(2Ca_3P_2O_8\,.\,CaO)\,.\,6CaO\,.\,3SiO_2$$
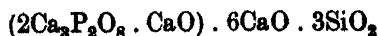
has also been produced by calcining Tunisian phosphate." The ratio in the 2nd citric extract again corresponds to the phosphate $4Ca_3P_2O_8 . 3CaO$, whilst the ratio in the 4th and 5th extracts corresponds to the phosphate $2Ca_3P_2O_8 . CaO$ present in Makatea Island phosphate. It is probable that the 3rd citric extract contains the phosphate $3Ca_3P_2O_8 . 2CaO$ present in calcined Belgian and calcined Gafsa phosphate.

The results obtained by submitting 5 grams of apatite to consecutive half-hour extractions with 2 per cent. citric acid are given in the following table.

Apatite, $3Ca_3P_2O_8 . CaF_2$.

Passes "100" sieve

	% P_2O_5	% CaO	Ratio $P_2O_5 : CaO$
1st citric extract	1·28	4·58	1 1·33*
2nd .. ,,	1·70	2·22	1 1·31
3rd ,, ,,	1·47	1·92	1 1·31
4th ,, ,,	1·47	1·88	1 1·28
5th ,, ,,	1·30	1·74	1 1·33
Extract totals	7 22	12·34	1 1·31
Actual extracts	39·49	55·45	1 1·33

* After subtracting 2·84 per cent. CaO present as $CaCO_3$.

These results show that the phosphoric acid and lime are dissolved out in the same ratio as they are present in the mineral.

CONCLUSIONS.

Five different mineral phosphates have been examined and the results secured indicate the presence of three distinct compounds of phosphoric acid and lime.

Name of phosphate	Nature of the phosphate present
Makatea Island	$2Ca_3P_2O_8 . CaO$; or $CaO{<}\begin{matrix} Ca_3P_2O_8 \\ \| \\ Ca_3P_2O_8 \end{matrix}$
Florida Pebble ⎫ Tunisian ⎬	$4Ca_3P_2O_8 . 3CaO$ $\begin{matrix} CaO{-}Ca_3P_2O_8 \\ \| \\ Ca_7P_6O_{17} \\ \| \\ CaO{-}Ca_3P_2O_8 \end{matrix}$
Algerian ⎫ Gafsa ⎬ Belgian ⎭	$Ca_3P_2O_8 . CaO$
Apatite	$3Ca_3P_2O_8 . CaO$

The substitution of fluorine or chlorine for the oxygen of one of the CaO groups in each of the above rock phosphates does not interfere with the ratio $P_2O_5 : CaO$. The combination of fluorine or chlorine in this manner would account for the low solubility of rock phosphates in citric acid compared with bone meal, whilst the higher lime content of the rock phosphates accounts for their higher solubility in citric acid compared with apatite[1].

The results of calcining the various mineral phosphates show that a citric soluble silica phosphate is formed. This phosphate goes into solution in the 1st citric extract. In addition to the silica phosphate one or more phosphates with a lower lime content than the original phosphate are produced by calcining. The longer the calcining continues the greater the tendency to produce phosphates of low lime content, and hence the lower the solubility.

It is of interest to note that with one exception (due probably to a higher fluorine content) the higher the percentage of lime actually combined with phosphoric acid the more soluble the mineral phosphate is in citric acid. Collins[2] has shown this to hold true in the case of basic slag.

[1] The Author. *Journ. Soc. of Chem. Industry*, 29 Feb. 1916, xxxv.
[2] *Journ. Soc. of Chem. Industry*, 29 May 1915, xxxiv.

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PASTURE PROBLEMS: INDIGENOUS PLANTS IN RELATION TO HABITAT AND SOWN SPECIES.

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I. INTRODUCTION.

IT is proposed in the present paper to trace (a) the relationship that exists between the several indigenous plants that contribute to the herbage of definite types of grassland; (b) to follow the progressive changes that occur on fields, down to grass for a varying number of years, belonging to these types; (c) to follow the competitive interaction between sown and indigenous species; and (d) to contrast the effect on the herbage of continual mowing and continual grazing.

The study of indigenous plants is an essentially local one, consequently the data here presented are derived almost entirely from our investigations in South and Mid Wales[1], occasional examples only being given from North Wales and the Cotswolds for the sake of comparison.

A list of books and articles that we have consulted, as well as those actually referred to in the text, is given at the end of the paper.

II. METHODS OF STUDY.

It is necessary first to recognise the salient types of grassland for the area investigated and then, by employing a qualitative and quantitative method of analysis, to obtain a numerical statement of the various changes under review. The methods of analyses employed

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[1] The subject has been briefly treated by Stapledon (15) elsewhere, and by Jenkin in an unpublished thesis ("The Persistence of Sown Grasses and other Plants in Pastures") presented for The Honours Degree at Aberystwyth, 1914.

have been those described by one of us in a previous paper[1](16). Comparisons are made, as far as possible, between figures obtained by the same method; but this is not always practicable when contrasting meadows with pastures. The actual method employed is always stated in the tables; and elsewhere mentioned in footnotes.

III. TYPES OF GRASSLAND INVESTIGATED.

At the outset it is necessary to distinguish between natural types and semi-natural types.

By natural types are here to be understood those which historical evidence suggests have never been extensively under the plough or manured; and which, if broken or manured at a remote period, have completely reverted to type. By semi-natural types[2] those which have certainly, at one time or another, been under the plough and, at all events, manured during the rotation previous to reverting to grass.

The semi-natural types may be further classified thus: *Untended*, those which have been ploughed and probably manured 50 to 100 years ago, and then, after yielding crops for some seasons, allowed to revert to grass without any sowing[3], and have so remained ever since, in most cases, without any further application of manures. *Tended*, those which have been down to grass about 20 to 50 years, in most cases, receiving periodic if but slight dressings of manure (at least during the earlier years) and probably sown in the first instance with rye grasses and clovers only, or with loft sweepings.

The following types, details of which are given hereunder, form the basis of the present investigations:

NATURAL TYPES.

The Board of Agriculture's returns for 1914 gave 21 % of Welsh land as being under mountain and heath vegetation. Much of this is *Molinia* and *Nardus* grassland and moorland vegetation which is

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[1] "Specific Frequency," the number of times a species (without regard to the number of plants of that species) occurs per 100 readings within a mesh 6″ × 6″. "Specific Productiveness"—the percentage of edible herbage arrived at by cutting, sorting, and weighing. "Percentage Frequency," arrived at by the number of plants of each species per unit of area. Jenkin has, however, used as his unit the number of individual "tillers" instead of individual plants.

[2] These would seem to correspond to the "Artificially Induced Grasslands" of Smith and Crampton (13).

[3] Excepting possibly some sweepings from the hay lofts.

not brought under the plough to any very appreciable extent, and consequently does not here primarily concern us. A large proportion, however, consists of heath (*Fescue*) grassland[1], considerable tracts of which have, from time to time, been brought under cultivation and again let down to grass; thus affording an excellent opportunity for the study of the gradual processes in the restabilisation of disturbed land. Two types of natural fescue pastures can be recognised:

1. *Mountain fescue pasture* (1400'–2500'). This is intermediate between the mountain *Nardus* pastures and the heath fescue pastures. It consists of a very restricted flora and if associated with gorse or bracken, these plants are isolated or in small clumps only; the gorse always being stunted specimens of *Ulex Gallii*. The Leguminosae are unrepresented in the ground flora. The chief grasses, with their cardinal figures[2] of occurrence, are *Festuca ovina* (21. 41. 56), *Agrostis vulgaris* with *A. pumila* (0. 3. 11), *Triodia decumbens* (0. 14. 39).

Distributed grasses (*i.e.* such as contribute nothing appreciable to the edible herbage) are *Anthoxanthum odoratum*, ·*Deschampsia flexuosa*[3] and *Aira praecox*[4].

The miscellaneous plants contribute from 1 % to 5 %; *Potentilla erecta* and *Galium saxatile* often being the only representatives of the heath herbs over considerable areas. *Luzula erecta*, *L. campestris*, *Carex binervis* and *Polygala* spp., are, however, usually distributed plants, whilst *Viola lutea*, *Hieracium Pilosella* and *Veronica officinalis* are occasional. *Juncus squarrosus* sometimes, especially at the higher elevations, occurs in considerable gregarious patches; but is usually accompanied by *Nardus stricta* and betokens a gradual passing into one of the *Nardus* types.

2. *Undisturbed heath fescue pasture* (600'–1700')[5]. Differs from the above in several important respects. Gorse and bracken are abundant in masses up to 900'–1000' on the Plynlimon area and up to 1500' on Radnor Forest and elsewhere.

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[1] For instance, 30 % of an area of 66,700 acres of mountain and heath-land behind Aberystwyth consists of heath grass land.

[2] See (17). Three figures in brackets given after a species represent (from left to right) its minimum, optimum, and maximum figures of distribution obtained by the method of Percentage Frequency.

[3] Most usually on acid flushes.

[4] On bare peaty places in conjunction with *Rumex Acetosella*.

[5] The altitudinal limit of this type varies considerably for different districts; it seldom exceeds 1200' in the Plynlimon area but in Brecon and Radnor frequently reaches 1700'.

TABLE I. *To contrast the average specific frequencies of typical miscellaneous plants on undisturbed and disturbed heath - fescue pastures and on permanent pastures derived from heath at the same elevations.*

The figures are the average of three analyses on each type. The following letters denote the results of more general observations:
o = occasional, l = local, la = locally abundant, g = gregarious.

Species at 700'–1100'	1 Undisturbed heath fescue pasture	2 Disturbed heath fescue pasture	3 Permanent pasture ex heath
HEATH HERBS PROPER:			
Viola lutea et var. amoena ...	4	10	o
Polygala spp.	20	20	o
Hypericum pulchrum ...	o	1	—
Potentilla erecta	90	56	10
Galium saxatile	96	40	o
Hieracium Pilosella ...	4	50	20
Jasione montana	2	3	—
Erica cinerea ...	1	1	--
Veronica officinalis ...	17	46	o
Euphrasia spp.*	o	1	l
Pedicularis sylvatica ...	2	4	—
Thymus Serpyllum ...	1	1	—
Stachys Betonica	o	3	—
Luzula campestris	23	18	19
L. erecta†	o	—	—
Carex binervis† ⎫			
C. praecox† ⎬	3	1	o
C. pilulifera† ⎭			
INTERMEDIATE HERBS:			
Cerastium spp.	o	1	20
Linum catharticum ...	—	o and l	13
Pimpinella Saxifraga ...	o	10	6
Leontodon autumnalis .	‡	o and l	5
TENDED PASTURE HERBS PROPER:			
Ranunculus repens... ...	—	2	95
Alchemilla vulgaris .	—	—	la
Daucus Carota	—	—	l
Bellis perennis	--	o	10
Cirsium arvense	-	—	g
Leontodon hispidus ...	-	—	l
Hypochoeris radicata ...	‡	3	20
Taraxacum officinale ...	—	—	20
Bartsia Odontites	—	—	la
Prunella vulgaris	‡	10	25
Plantago lanceolata ...	‡	15	90
Rumex Acetosa	—	—	10
Cardinal figures for total (percentage frequency) contribution of miscellaneous herbs	(3. 9. 20)	(5. 11. 27)	(8. 18. 30)

* More plentiful on the undisturbed heaths on Radnor Forest and Brecon Beacons

† More plentiful on the mountain fescue pastures.

‡ Occasionally met with near sheep tracks, fences, and roads.

The gorse is *Ulex Gallii* except occasionally at the lower elevations where *U. europaeus* also occurs.

Lotus corniculatus and *Lathyrus montanus* are slight contributors to the herbage but together never exceed 2 %. The grasses are the same as on the mountain type but have decidedly different distributions as the following cardinal figures show:

Festuca ovina (15. 32. 49), *Agrostis vulgaris* and *A. pumila* (7. 14. 25), *Trioda decumbens* (5. 9. 20). The most noteworthy feature of this type is, however, the number and abundance of characteristic herbs. These are given together with their average specific frequencies in Table I.

SEMI-NATURAL TYPES

A. *Untended: under crops 50-100 years ago.*

1. *Upland disturbed heath fescue pasture* (600'-1300'). This type, as well as being generally recognisable by vestiges of the narrow lands in vogue a generation or so ago, differs in its herbage from the undisturbed fescue pasture in several diagnostic respects.

Gorse and bracken are abundant and frequently contiguous over large areas, *Ulex europaeus* being, especially at the lower elevations, as plentiful as, and locally more plentiful than *U. Gallii*[1]. The Leguminosae are here appreciably represented in the ground herbage; *Lotus corniculatus* and *Lathyrus montanus* are of general occurrence. *Trifolium repens* is frequent and sometimes contributes as much as 8 % to the herbage; *T. pratense* is an occasional and local plant. The cardinal figures for the four leguminous herbs together are (1. 4. 10). The chief grasses are again *Festuca ovina* (18. 28. 50). *Agrostis vulgaris* (10. 20. 48) and *Triodia decumbens* (2. 5. 16), from which it is seen that the average contribution of *Agrostis* on this type is but little short of the *Fescue*. *Cynosurus cristatus* sometimes occurs in appreciable amount and is frequently a distributed grass, whilst *Festuca rubra* finds a suitable habitat amongst gorse bushes and under the shade of distributed bracken. The miscellaneous herbage gives a decided character to this type; for not only do the heath herbs proper give strikingly different frequencies on the disturbed and undisturbed

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[1] The greater local abundance of *U. europaeus* may be due to the fact that the seed of this plant has been largely sown in the past; this is the more likely since sporadic plants of *Echium vulgare* are sometimes met with amongst the *Ulex*. The seeds of this plant may, in the first instance, have been introduced with the *Ulex*.

pastures, but a number of plants, typical of tended grasslands, are met with on the disturbed fescue pasture (see Table I).

2. *Lowland disturbed heath fescue pasture* (50′–600′). Small areas of heath occur at these lower altitudes, usually comparatively near the sea[1] or on the tops of the higher hills in the zones of cultivated land proper. Some of these have doubtless never been ploughed but since they are nearly all grazed by stock having periodic access to tended and young pastures, they are considerably interfered with, and, consequently, in most cases, appertain more to the semi-natural than the natural[2]. Gorse and bracken are as usual abundant, *Ulex europaeus* being now as or more plentiful than *U. Gallii*. The Leguminosae (5. 15. 33) are considerably more plentiful on the ground flora than on the foregoing types; this is due to the predominance of *Lotus corniculatus* and *Trifolium repens*. *Trifolium pratense*, *T. minus* and *Vicia Cracca* are frequent, whilst *Lathyrus pratensis* is more plentiful than *L. montanus*. The chief Gramineae are as follows: *Agrostis vulgaris* (with excess of *A. pumila* near the sea) (20. 22. 30), *Festuca ovina* (10. 16. 20), *F. rubra* (5. 8. 17), *Triodia decumbens* (2. 5. 8), *Cynosurus cristatus* (0. 4. 13), and *Anthoxanthum odoratum* (up to 5 %). *Lolium perenne*, *Holcus mollis* (especially near woods), *Holcus lanatus*, and *Poa* spp. are sometimes distributed plants. It is interesting to note that *Agrostis vulgaris* is here the predominant grass, being often more plentiful than *Festuca ovina* and *F. rubra* together, and that *Cynosurus cristatus* contributes satisfactorily to the herbage. The miscellaneous plants are again represented by the heath herbs mentioned in Table I; *Galium saxatile* and *Potentilla erecta* do not, however, constitute the predominant element in the included flora, seldom attaining to higher specific frequencies than 30. and 25. respectively. Most of the "Intermediate" and "Tended pasture" herbs of Table I are here normal constituents of the vegetation, and, in addition, the following are of general or local occurrence:

Lepidium Smithii (especially near the sea)
Sagina procumbens (frequent)
Daucus Carota (occasional)
Solidago Virgaurea (especially near woods)

Crepis virens (frequent)
Leontodon hispidus (locally abundant)
Carlina vulgaris (near the sea)
Senecio Jacobaea (in the absence of sheep grazing)

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[1] Heath pastures also occur on the cliffs quite close to the sea; these, however, have characteristics of their own and need not be considered here.

[2] Evidence, however, suggests that further investigation will make it possible to differentiate between natural and semi-natural types in the case of these somewhat restricted heaths also.

The cardinal figures for total miscellaneous plants are (7. 15. 30) which compared with (1 to 5) on mountain fescue, (3. 9. 20) on undisturbed heath fescue, and (5. 11. 27) on disturbed heath fescue show a progressive increase on the fescue types from high to low elevations.

B.   *Tended: under crops* 20–50 *years ago.*

It will be convenient firstly to consider the types that occur on the above-mentioned heath lands; that is to say on land that if neglected long enough would revert *via* the untended types to mountain fescue pasture, upland heath fescue pasture, or lowland heath fescue pasture according to the altitudes.

Secondly to deal with types which occur on alluvia, gravel, or glacial drift, types which under prolonged neglect would not revert to heath fescue pastures.

1. *Permanent pastures derived from heath fescue types.* These pastures are to be met with on the small "intaken" farms on the heath zone and on the larger farms at the higher elevations of the main cultivated tracts. The soils are usually thin and somewhat sticky, being, in the case of most of the fields studied, derived from Terannon Shales. It is sufficient for the purpose of the present paper merely to differentiate between two main types, namely, those which occur respectively (*a*) above and (*b*) below about 600′–700′.

(*a*) *Permanent pasture above* 600′–700′. This differs from the disturbed heath fescue pasture in three respects: (1) the far larger and floristically more varied contribution of the Leguminosae to the total herbage; (2) *Agrostis vulgaris* is usually more plentiful than *Festuca ovina,* and a number of pasture grasses are appreciable contributors to the herbage; (3) the predominance of the more typical weeds of tended grassland over the heath herbs and the greater aggregate contribution of the miscellaneous plants. This is seen by reference to Table I. *Centaurea nigra* and *Rhinanthus* spp. are also very abundant.

The chief contributing grasses and clovers with their cardinal figures are as follows:

Festuca ovina (2. 15. 30)

F. rubra (0. — 15)

Agrostis vulgaris (4. 20. 70)

Triodia decumbens (2. - - 6)

Holcus lanatus* (2. 10. 20)

Anthoxanthum odoratum (1. 3. 10)

Lolium perenne† (2. 3. 8)

Cynosurus cristatus (2. 6. 5)

Poa trivialis distributed

Poa pratensis distributed

Phleum pratense   „

Dactylis glomerata distributed

Bromus mollis et spp. rare

Trifolium repens

T. pratense

Lathyrus pratensis } (1. 16. 43)‡

Vicia Cracca

Lotus corniculatus

* The maximum figures are recorded under meadow conditions.

† The maximum figures are recorded when farmyard manure is freely applied.

‡ The maximum figures are recorded when phosphatic manures have been recently added, and lime applied to the soil.

(b) *Permanent pasture below* 600'–700'[1]. This is broadly differentiated from the above by a considerable falling off in *Festuca ovina*, its place being largely taken by *F. rubra*, the two together, however, seldom reaching 20 %. *Agrostis vulgaris* may as completely overrun pastures at these lower elevations as above 600'; but usually it is decidedly less plentiful. *Cynosurus cristatus* to some extent takes the place of *Agrostis* and seldom has a frequency of less than 5 %. *Lolium perenne* (4. 6. 8) also has higher cardinal figures at the lower than higher elevations. *Poa trivialis* and *Poa pratensis* are usually appreciably represented; the former may reach 6 %. *Dactylis glomerata* occasionally reaches 4 %, whilst *Phleum pratense* and *Bromus mollis* are distributed grasses. The Leguminosae seldom fall short of 5 %. Amongst miscellaneous plants the points of difference are slight; *Daucus Carota, Heracleum Sphondylium* (not abundant on pastures well grazed with mixed stock), *Crepis virens, Carduus lanceolatus* and *Rumex crispus* are, however, on the average, more plentiful at the lower elevations.

2. *Permanent pastures on alluvia, gravel, and glacial drift.* In the districts under review the amount of alluvium and gravel met with above about 300' is relatively insignificant; glacial drift, however, is abundant up to the highest limits of heath and mountain cultivation.

The natural grasslands on the glacial drift are of the *Molinia* and *Nardus* types; the permanent pastures above about 400' are intermediate in character between those described above (on Terannon Shales) and those met with on drift at lower elevations. The pastures

[1] The contour line which actually separates this from the foregoing type varies somewhat widely from one locality to another; in the districts under review, however, the line of demarcation usually falls within the limits given above.

on drift usually having a slightly higher percentage of *Lolium perenne* and *Poa trivialis* than their counterparts on shales, whilst *Ranunculus acris* and *Lotus major* are generally included in the flora.

It will be sufficient here to draw two main distinctions between the lowland pastures (below 200'–300') on alluvia, gravel, or drift:

(*a*)   Permanent pastures on well-drained alluvia or on gravel.

(*b*)   Permanent pastures on ill-drained alluvia or on drift.

(*a*)   *Permanent pastures (below* 300') *on well-drained alluvia or gravel.*   This type is the equivalent (although much poorer) of the famous grasslands of Leicestershire and Northamptonshire, and those locally met with in Somersetshire and other counties.   There is little or no difference to be seen in the herbage between those 20–50 and 50-100 or more years down, provided they are manured and stocked on a uniform plan.   It is difficult therefore to decide if these should be classed as natural or semi-natural.   In these districts, however, the evidence goes to show that when neglected they do actually alter in the direction of semi-natural to natural types, that is to say in an elimination of species.

In practice the best are seldom neglected : they are heavily grazed with stock assisted with cake and manured at regular intervals— consequently the husbandry becomes a predominant and constant environment factor which largely counterbalances purely natural tendencies and makes for a secondary stability[1].   The effect of this constant factor of judicious husbandry is to maintain a favourable ratio of desirable indigenous herbage (*Lolium perenne, Poa trivialis*, etc.) to undesirable (*Agrostis alba* and weeds).   In the districts under review on well-tended fields the chief contributing species with their cardinal figures are :

Lolium perenne (3. 9. 22)	*Agrostis alba* et vars. (3. 15. 25)
Poa trivialis (1. 4. 9)	*Phleum pratense* distributed
P. pratensis (1. 3. 8)	*Alopecurus pratensis* local
Festuca rubra (2. 5. 13)	*Bromus mollis** et spp. local, occasion-
Cynosurus cristatus (6. 9. 11)	ally reaches 17
*Dactylis glomerata** (2. 6. 20)	Leguminosae chiefly *Trifolium repens*
*Holcus lanatus** (2. 7. 13)	(8. 19. 36)
Anthoxanthum odoratum (2. 4. 9)	Miscellaneous plants (3. 9. 17)

* The maximum figures are reached under meadow conditions.

Comparison of the above synopsis with that given for permanent pastures derived from heaths, shows that *Agrostis alba* has definitely

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[1] Cf. Smith and Crampton (13), p. 4.

taken the place of *A. vulgaris*, whilst *Festuca rubra* has almost completely replaced *F ovina*. *Lolium perenne, Poa* spp. and *Dactylis glomerata* now contribute substantially to the flora. *Trifolium repens* is abundant and *Lotus major* is a distributed plant. Amongst the miscellaneous herbs a number of species are met with as distributed plants which are quite exceptional on the ex-heath types, *e.g. Ranunculus acris, Cardamine pratensis, Carex ovalis,* and *Ophioglossum vulgatum,* whilst on river side alluvia in Radnor and Brecon *Poterium officinale* is a characteristic plant.

(b) *Permanent pastures (below 300') on ill-drained alluvia or on glacial drift.* This type differs from that on well-drained land chiefly in the Leguminosae and miscellaneous herbs. *Lotus major* becomes a characteristic and often abundant plant; whilst *Trifolium pratense* is not plentiful. The chief point in the miscellaneous flora is the advent of such typical monocotyledons as *Juncus conglomeratus, J. effusus,* and *J. articulatus*[1], and a number of *Carices* of which the following are the most general: *Carex panicea, C. echinata, C. flava, C. Goodenovii,* and *C. pulicaris.* A number of typical dicotyledons are also proper to this type, *e.g. Caltha palustris, Spiraea Ulmaria, Potentilla palustris, Lychnis Flos-Cuculi* and *Ranunculus Flammula.*

The gramineous herbage is not very different from that on the well aerated lands, but *Deschampsia caespitosa* is here a frequent addition, and *Briza media,* a very rare plant in this district, has only been met with on this type[2]. *Agrostis alba* tends to become very plentiful, whilst the true *A. stolonifera* is more frequent here than elsewhere, and *A. alba* var. *coarctata* Hoffm. is to be met with on this type near the sea. *Molinia caerulea, Nardus stricta* and *Triodia decumbens* are also occasional plants.

The essential difference between the grassland on well- and ill-drained land is well seen in the case of rush-sedge pastures which have been almost completely neglected for 50 years and upwards. These then show unmistakable evidence of a return to the more stable moorland vegetation. The Leguminosae fall off considerably being usually only represented by *Lotus major* and *Genista tinctoria*; the dominant grasses become *Molinia caerulea, Nardus stricta,* and *Triodia decumbens, Agrostis*

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[1] *J. articulatus* is often relished by stock (in particular dairy cattle) especially in spring when it is tender; and on some experimental plots in Anglesey where the plant was growing on peat, it was much encouraged by phosphatic manures.

[2] *Briza media* is however a fairly common plant on calcareous loams in Pembroke-shire, on the Cotswolds and in N. Wales.

alba et *A. canina*. Additional plants are *Carex curta, Luzula* erecta and *L. congesta, Scabiosa Succisa* and locally *Eriophorum angustifolium*.

Neglected pastures on well-drained land pass, however, directly into scrub with *Prunus spinosa, Crataegus Oxyacantha*, and *Rubus* spp.; whilst intermediate cases are frequent which pass into alder holts with the addition of *Molinia* and other plants mentioned above.

IV. THE LATER STAGES IN THE STABILISATION OF GRASSLAND.

It is only possible to study the relationships of indigenous species on old grasslands (natural and semi natural) and the later stages in the stabilisation of such pastures by a comparison of types and by a series of deductions. The conclusions to be drawn are as follows:

(1) That the grasslands of this country conform to a number of well-marked types.

Our natural grasslands have been largely studied by ecologists and have been partially classified[1]. The factors which favour the development of grassland and which make for one type rather than another have been investigated by Smith and Crampton (13).

Carruthers (5)[2] in his work on tended grasslands clearly brought out in his published data the fact that semi-natural types could be recognised. He was, however, chiefly concerned with the agricultural value of species *qua* species and consequently did not elaborate this aspect of the problem and was himself rather impressed with the ubiquity of the ordinary grasses and clovers. Smith and Crampton (13), furthermore, have remarked upon the apparent lack of connexion between habitat and variations in their "Artificial" grasslands. It will, however, be apparent from a consideration of the types here reviewed that the tended classes of semi-natural grasslands differ from each other qualitatively chiefly in respect of the included miscellaneous flora and the relatively unimportant members of the Leguminosae and less in respect of the ordinary agricultural grasses and clovers, which albeit exhibit very considerable quantitative differences. There is, none the less, rather more qualitative variation amongst the Gramineae on the poorer types of semi-natural grasslands found within Watson's agrarian zone than Carruthers' observations would seem to have suggested.

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[1] See *e.g.* (12) and (19).
[2] And to a less extent Fream (7) and (8).

(2) The more stable grasslands, *i.e.* those which have probably not been under cultivation in historical memory, are colonised by relatively few species; whilst on pastures down no longer than about 20 years the number of contributing species is considerable.

Thus the end stage in stabilisation of grasslands is reached when the few most enduring species have suppressed the larger number of less successful ones.

Plants may therefore be considered as primary indigenous species when they colonise land which has never been disturbed or which has become completely re stabilised; the number of such plants (in so far as grassland is concerned) is relatively few. Examples are, *Molinia caerulea* and *Nardus stricta* and *Juncus squarrosus* on moorland and allied grasslands. *Festuca ovina, Agrostis vulgaris, Triodia decumbens,* and *Anthoxanthum odoratum* (as a distributed plant), with *Galium saxatile* and *Potentilla erecta* and other herbs on the Welsh hills. *Bromus erectus, Festuca rubra* var., *Lathyrus pratensis* and *Galium verum*[1] on the Cotswolds. *Agrostis alba* et vars., and to a slight extent possibly also *Lolium perenne, Poa* spp., *Cynosurus cristatus, Anthoxanthum odoratum* and *Festuca rubra* on certain old lowland permanent grasslands. (See Paragraph 5, hereunder.)

(3) Plants are secondary indigenous species when they appear (by themselves without sowing) on disturbed land. In a sense these plants are followers of man. They tend to come in on land that has been ploughed, very heavily manured, or even heavily grazed by stock near a homestead. The effect of disturbance has presumably been to upset the equilibrium between habit and primary species in a manner favourable to the secondary species which are no longer suppressed by their primary competitors. The secondary indigenous species may furthermore persist for a generation even after man has entirely withdrawn his attention. Most of the ordinary herbage plants[2] are secondary species on the majority of tended grasslands, several being confined to definite types, and all showing a decided quantitative relation to habitat; and thus, in conjunction with the primary species, they give a definite character to the various types of semi-natural grassland.

(4) The altitudinal limit of a species depends largely on competitive interaction; and since the secondary species are finally suppressed by

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[1] Also a locally prominent plant on some of the Radnorshire hills.

[2] The valuable grasses and clovers of the Agricultural text books, and the miscellaneous plants of tended grasslands.

the primary, they occur at higher elevations where land has been disturbed (even 50–100 years ago) than elsewhere. This is well illustrated by a comparison of the disturbed with the undisturbed heath fescue pastures (of the uplands) which have very similar altitudinal limits, yet the disturbed have a more varied flora than the undisturbed. Again, *Vicia Cracca, Crepis virens.* and *Leontodon hispidus* are more or less common on the semi-natural untended heath pastures below 600′; but have not yet been met with on the corresponding type above 600′; yet they have been frequently seen on the more recently disturbed and less stable permanent pastures (20-50 years down) at even 1000′; whilst *Hieracium boreale,* a plant not uncommonly met with on the heaths below 600′, but, as far as our observations go, absent from those above, has once or twice been seen on old tended grasslands at 900′–1000′. *Trifolium repens,* normally absent from the natural heath pastures, has been found at 1900′ on sheep tracks on Radnor Forest and elsewhere under similar conditions at 1700′.

(5) The delaying effect of grazing animals on the progress of stabilisation cannot be overlooked. Smith and Crampton (13) have pointed out that but few of our grasslands are stable (in the ecological sense of the word), but that the majority are migratory and tend t pass into moorland or woodland types of vegetation, and that th changes are delayed by the continued operation of animals. Th neglected[1] "tended pastures" at the edges of woods may revert rap to scrub without going through the more normal intermediate sta of disturbed to undisturbed heath.

It would seem probable, moreover, that the grazing factor influences all the gradual stages in stabilisation that grasslands themselves pass through.

Thus the difference between the mountain fescue pastures and the undisturbed heath fescue pastures must not be regarded as only due to more rigorous climatic conditions (due to difference in altitude); for since the latter tend to offer more favourable grazing grounds for sheep, this factor of greater interference by animals may in part account for the more varied flora (more backward stabilisation) of the heath than the mountain pastures. Again, on sheep walks that are very understocked, even at comparatively low elevations, the tendency is for the fescue types of grassland to pass into the *Nardus* types. Furthermore, constant and heavy grazing on the best types of permanent grassland has been shown to be, almost certainly, competent to occasion a state

[1] Very understocked.

of secondary stabilisation and thus to give to a number of plants which are probably only secondary indigenous the semblance of primary species on such habitats (*e.g. Lolium perenne, Poa* spp., *Cynosurus cristatus*, and *Dactylis glomerata,* which, if truly primary, are probably only so as distributed plants).

From these considerations it follows—since the more valuable plants are usually secondary species and occur under conditions of delayed stabilisation—that reasonably heavy stocking favours the development of desirable pastures.

Provided therefore that lime and phosphatic manures are adequately applied to compensate for the removal of these ingredients from the soil, our sheep walks and the majority of our grasslands could be enormously benefited by carrying a greater head of stock[1].

(6)   A comparison of all the heath fescue types above 600′, including permanent grassland (over 20 years down) on land derived from heath, illustrates very well the progressive stages in the competitive interaction 'tween primary and secondary species towards the end stages of grass-nd stabilisation[2].   They are as follows:

At 20–30 *years* (permanent grassland above 600′, p. 32).

Under the influence of periodic manuring and comparatively heavy grazing with cattle and sheep the secondary species (*e.g. Cynosurus cristatus, Trifolium repens, Plantago lanceolata,* etc.) are still abundantly represented; the primary species (*e g. Agrostis vulgaris, Festuca ovina,* and the heath herbs) are, however, beginning to take a prominent place in the herbage.

At 50–100 *years* (disturbed heath fescue pasture, p. 30)

Under the influence of a discontinuance of manuring and of grazing chiefly with sheep, the primary species have gained considerably but do not yet stand in their normal relation to each other; the secondary species are much reduced.

At over 100 *years* (undisturbed heath fescue pasture, p. 28).

The influence of the original disturbance and of manuring is now finally lost; the grazing is generally by sheep only, the nett result being that the primary species have completely suppressed the secondary species.

[1] A just balance between the potentialities of the herbage under the influence of manures and a gradual increase in the stocking, and the limits set to the number of stock carried due to pathological influences of over-crowding, is all too seldom the ideal of graziers.

[2] Stabilisation in so far as the fescue types of grassland are concerned.   We are not here concerned to decide if the mountain fescue is an ecologically stable type.

Final stabilisation (mountain fescue pasture, p. 28).

Under the combined influences of more rigorous climatic conditions and a further falling off in the number of grazing sheep, elimination of species has gone farther; the primary Leguminosae and several heath herbs being now absent from the flora.

It therefore follows that, in the case of the fescue types, final grassland stabilisation is only reached when all the Leguminosae, a great majority of the miscellaneous herbs, and most of the grasses, have been completely suppressed, and when *Festuca ovina* has regained a dominant position at the expense of *Agrostis vulgaris*.

The more rigorous climatic conditions at the higher altitudes make for final stabilisation and consequent mountain fescue pasture; all gradations are, however, to be seen at the upper limits of the undisturbed fescue pasture between it and the mountain type. Similar gradations are also frequently met with even below the normal line of demarcation between the two; and at the lower elevations (below 600′) small areas may similarly be encountered with a much restricted flora. It is therefore not improbable that the heath types are actually in a state of slow reversion towards the more stable mountain type just as the tended types revert to untended types and then the disturbed to undisturbed heaths. If this is the case, it follows either that (*a*) stabilisation becomes a slower and slower process as the climatic conditions become less rigorous at the lower altitudes and as the grazing becomes heavier and more influenced by proximity to tended lands and the homestead; or (*b*) the undisturbed fescue pastures have been actually ploughed or otherwise considerably interfered with by man in a very remote past.

(7) The types on well- and on ill-drained alluvia and on boulder clay illustrate the same phenomena. On the ill-drained soils stabilisation results in a dominance of the primary species (*Molinia caerulea, Nardus stricta,* and *Agrostis* spp.) with an ultimate elimination of the secondary ones (*Trifolium* spp. and sundry herbs). On the well-drained soils also the number of gramineous species is decreased and *Agrostis alba* tends to become dominant; but the distinction between primary and secondary species is not so well marked as on other types.

V. THE EARLY STAGES IN THE STABILISATION
OF GRASSLAND.

These can be accurately studied on fields of which the agricultural history is well known from the first year they are put down to grass until they are 10-20 years old.

Middleton (11) has pointed out the gradual stages in the development of a pasture, which passes from youth through middle to old age. It will be shown that youth constitutes the period when the sown species are competing with arable land weeds and the secondary indigenous plants; middle age when the more persistent sown species are competing with the secondary plants and with the primary species; and old age when the secondary and primary plants have almost completely dominated the herbage. Thus, when old age is reached, a tended pasture has approximated very closely to the semi-natural.

Thompson (20) has observed that the first year's produce from sown seeds is usually the best, and that after the second year (middle age) there is a marked falling off. On poor soils especially the middle age is the critical time; and unless the management is good the middle period will commence with the second year; on good soils and under clever management, however, this period can be delayed and rendered less obvious.

If a field belonging to a well-marked type (*e.g.* disturbed or undisturbed heath fescue pasture) is ploughed and but one crop of corn taken and then allowed to run down to grass without seeding it will not immediately revert to its original indigenous characteristics.

For instance on the Cotswolds a field run down from a wheat stubble in 1908 was observed in 1911 to be still unrepresented by such fundamental primary species (for that district) as *Bromus erectus*, *Galium verum*, and *Carduus acaule*: while *Carex glauca* was considerably below its normal development. In Mid-Wales on the heath types above 600' *Agrostis vulgaris* has on such fields frequently been observed to stand in a higher ratio for at least six years to *Festuca ovina* than it does on the undisturbed heaths. Thus, when a field is disturbed, to the extent of but one crop, we see that most, but not necessarily all, of the primary indigenous species rapidly re-colonise the ground, but that several years (probably 6-12) must elapse before all the fundamental primary species are again represented and conform to their cardinal figures for the original type. It is, however, when fields have been put through the complete rotation and then sown with rye grasses

and clovers or with a more complete mixture that all the stages in stabilisation can be best followed.

It is not necessary for the purpose of tracing the main features in the competition of sown with un-sown species to differentiate between all the types of grassland previously discussed. It will be sufficient to deal with (A) Fields at and above 600', which may occur on either shales or on clay; (B) Fields below 600' on shales; and (C) Fields below 300' on well-drained alluvia and on glacial gravel.

A. FIELDS ABOVE 600'.

The herbage ultimately covering such fields would depend upon the soil. If it was derived from shales then it would become a heath fescue pasture; or, if it was a drift clay, then a *Molinia* or *Nardus* pasture would finally result. The early stages are, however, very similar in both cases, and for the sake of brevity are classed together in some of the tables given. It will be convenient to deal firstly with fields sown only with perennial rye grass, red clover and trefoil; and secondly with fields sown with a more complete mixture.

Fields sown with rye grass, red clover and trefoil, only.

Over fifty fields have been analysed which were sown with the above simple mixture; some of the fields have received farmyard manure, some superphosphate, and some basic slag. In most cases hay was taken for the first one to four or six years; and the fields subsequently grazed. The results obtained are presented in a generalised form in Table II; the percentage range of occurrence for each species being given in terms of percentage frequency. Some further details are given in the footnotes directly under the table.

The chief points to be noted from these results, and from the data presented in subsequent tables, will be discussed under the several species in the summary at the end of this section.

Fields sown with a more complete mixture.

Two interesting sets of data are available for fields sown with comparatively complete mixtures. The fields were 650'–700' above sea level on thin soil over shale; in both cases farmyard and phosphatic manures were freely applied. In one case (see Table III) hay was taken for the first two years—the field being subsequently used only

TABLE II. *To show the range of percentage frequency of the species contributing to the herbage of fields down to grass 1–20 years; when Lolium perenne, Trifolium pratense, and Medicago lupulina only are sown at from 600'–760' above sea level.*

Hay or pasture	Hay	Hay	Hay	Hay	Hay or pasture	Pasture	Pasture	Pasture
Age of field	1	2	3	4	5–6	7–8	10–12	15–20
Lolium perenne	20–70	30–66	4–15	1–8	2–5	0–4	0–6	0–8
Holcus lanatus	2–5	2–42	30–60	14–44	18–27	2–15	2–6	2–6
Agrostis vulgaris	—	0–6	5–30	2–32	5–15	18–40	15–56	20–70
Bromus mollis	1–2	1–7	0–1	0–1	—	—	—	—
Cynosurus cristatus	—	0–1	0–6	0–20	0–15	3–20	8–20	4–20
Anthoxanthum odoratum	1–2	1–2	1–3	1–10	2–9	2–6	2–5	1–10
Festuca ovina	—	—	—	0–8	0–4	3–20	5–21	7–30
Triodia decumbens	—	—	—	—	—	1–7	1–6	1–6
Molinia or *Nardus*	—	—	—	—	—	0–2	0–4	0–25
Trifolium pratense	15–40	10–17	2–16	0–2	—	0–4	0–4	0–4
T. repens	—	—	0–11	0–20	0–15	0–30	0–15	0–10
Lotus corniculatus, T. minus Lathyrus pratense, Vicia Cracca	0–2	0–11	3–10	2–32*	2–33†–56*	9–20	4–10	4–9
Rhinanthus spp.	—	—	4	13–42	13–40	10–20	4–10	2–4
Ranunculus repens, Bellis perennis	2–10	6–15	10–17	3–14	9–27	8–26	8–28	6–30
Crepis virens, Plantago lanceolata, etc.	—	—	—	—	—	—	—	—
Galium saxatile Potentilla erecta, etc.	—	—	—	—	0–2	0–3	1–3	2–4

NOTES. *Lolium perenne* occurs in larger quantity after the third year on the clay soils ex drift than on the soils ex shales; *Cynosurus cristatus* also attains to its highest figures on the drift soils, and it is on these soils that *Molinia* or *Nardus* may appear. *Trifolium repens* is only found in considerable quantity where phosphatic manures are added; but it does appear on fields where these manures have not been applied. *Crepis virens* is frequently very abundant in the first hay crop. *Centaurea nigra* does not appear in any quantity until the fourth year.

* Chiefly *Trifolium minus*.   † Chiefly *Vicia Cracca*.

as a pasture. In the other case (see Table IV) hay was taken con&tantly for six years.

TABLE III. *To show mixture used in lbs. per acre and percentage frequency of the herbage on a pasture at 650'–700' in its fifth, sixth and seventh years respectively.*

Contributing Species	Mixture in lbs. per acre	5th year	6th year	7th year
Lolium perenne	12	6·6	6·2	5·4
Dactylis glomerata	3	2·1	1·4	0·7
Phleum pratense	3	1·5	0·2	0·3
Other grasses (sown)	8 *	0·3	0·2	1·1
Cynosurus cristatus		8·1	9·2	10·9
Festuca rubra		7·9	6·1	7·7
Agrostis vulgaris (et *A. alba*) ...		17·5	17·1	14·9
Anthoxanthum odoratum † ...		18·9	21·2	11·3
Holcus lanatus		2·5	2·6	5·0
Other grasses (not sown) ...		1·5	2·0	2·2
Trifolium repens	2	12·4	17·4	18·7
Other Leguminosae	5 ‡	0·3	0·7	0·9
Ranunculus repens		3·6	1·3	1·1
Bellis perennis		7·3	4·4	5·2
Prunella vulgaris		2·8	2·6	5·9
Achillea Millefolium		0·9	0·9	1·4
Other miscellaneous herbs ...		5·8	6·5	7·3

* *Lolium Italicum*, 5 lbs.; *Festuca duriuscula*, 2·5 lbs.; *F. elatior, F. pratensis* and *Poa pratensis*, 0·25 lb. each.

† The figures for this grass are unusually high; and are probably due to the introduction of the seed by wind from a piece of waste ground near the field on which the grass was very plentiful.

‡ *Trifolium pratense*, 2 lbs.; *T. pratense perenne*, 2 lbs.; *T. hybridum*, 1·5 lbs.; *Anthyllis Vulneraria*, 0·25 lb.

A number of other analyses are available of fields of various ages at these elevations sown with known mixtures. In order, however, not to unnecessarily burden this paper with tables, the chief results obtained can be briefly stated as follows:

Lolium Italicum. 4 and 5 lbs. sown has not given traces of the plant after four years.

Dactylis glomerata. On clay drift at 600', 3 lbs. per acre gave 5 % of herbage after four years; and 8 lbs. per acre gave 11 % of herbage. After eleven years the herbage from 6 lbs. was only 1·5 %.

Phleum pratense. On the average of two sets of analyses equal sowings of this grass on soil ex shale and ex drift (= clay) gave about the same percentage of herbage in both cases at four years.

Féstuca elatior. 4 lbs. per acre on clay only showed negligible results at eleven years.

Festuca pratensis. 5 lbs. per acre gave only 4–5 % at four years and a negligible quantity at eleven years.

Festuca duriuscula(?). 1 and 2 lbs. of the commercial seed sown on clay left no plants to be seen after four years. *Festuca rubra* although not sown gave 1 % to the herbage on shale and 2 % on clay at four years.

TABLE IV. *To show percentage productiveness of the hay crop for six years in succession at* 650'; *on fields sown with a mixture of* Lolium perenne, Dactylis glomerata (4 *lbs.*) *and* Trifolium pratense.

Species	Successive Hay Crops					
	1	2	3	4	5	6
Lolium perenne	38	21	13	8	3	4
Dactylis glomerata ...	2	10	36	46	45	9
Holcus lanatus	3	20	26	10	2	3
Anthoxanthum loratum ...	0	1	1	5	6	6
Cynosurus cristatus ...	0	0	0	3	12	17
Agrostis vulgaris ...	0	0	0	1	2	6
Trifolium pratense ..	25	19	traces	2	2	3
T. repens	—	10	8	7	7	4
T. minus	—		2	1	1	3
Miscellaneous herbs ...	32*	19†	14‡	17§	20§	45§

* Practically all *Crepis virens.*

† Chiefly *Ranunculus repens, Bellis perennis* and *Prunella vulgaris.*

‡ As † with some *Rhinanthus* spp. § Chiefly *Rhinanthus* spp.

Alopecurus pratensis. 3 lbs. on clay left nothing to be seen at the end of four years.

Cynosurus cristatus. 1 lb. on shale gave 43 % to the herbage after four years; 20 % being the highest figure recorded in the fourth year without seeding. 2 lbs. on shale gave 5 % to the first hay crop, when unsown this grass being negligible in the first year.

Poa trivialis. 1 lb. on clay drift gave after four years 14·8 % to the herbage and when not sown at four years it contributed 1·6 %.

Trifolium repens. 2 lbs. of commercial White or Dutch clover gave 8 % to the herbage on shale at four years, this being no more than frequently occurs when this seed is not included in a mixture. On clay 3 lbs. of commercial seed gave 13 % and 2 lbs. 7 % to the herbage at four years; as even the larger percentage is frequently

surpassed without seeding, the figures do not suggest that the larger seeding is necessarily advantageous.

Cichorium Intybus. 1–2 lbs. per acre only leaves traces after four years.

Poterium Sanguisorba. 3 lbs. on shale left traces after four years; 5 lbs. on clay drift left nothing to show after four years.

B. FIELDS BELOW 600′ (CHIEFLY ABOVE 200′) ON SOILS DERIVED FROM SHALE.

The data available are not so extensive as for the higher elevations. Over twenty-five fields have, however, been investigated; the results obtained from the use of a comparatively simple mixture are set out in Table V.

TABLE V. *To show seeds mixture used and the contribution to the herbage of the several species on fields from one to eight years down to grass at 400′–500′ above sea level. All the fields received farmyard and phosphatic manures.*

Contributing species	Seeds mixture in lbs per acre	Treatment and age of fields							
		Hay	Hay	Hay	2 years pasture after 1 hay crop	1 year pasture after 3 years hay	3 years pasture after 1 hay crop	4 years pasture after 1 hay crop	8 years continued hay
Age of fields...		1*	2*	2*	3†	4†	4†	5†	8*
Lolium perenne	16–20	40	30	40	10	12	6	8	5
Dactylis glomerata	3–4	8	15	4	2	14	3	3	--‡
Phleum pratense	2	—	10	2	2	1	trace	—	—‡
Cynosurus cristatus	—	—	—	—	—	—	—	6	30
Festuca rubra	—	—	—	—	3	2	—	1	3
Holcus lanatus	—	6	10	24	8	15	19	13	15
Agrostis vulgaris	—	—	15	6	6	2	35	41	1)
Poa spp.§	—	—	3	—	6	11	4	—	5
Anthoxanthum odoratum	—	—	—	—	—	—	—	trace	5
Bromus mollis	—	3	—	2	—	—	—	—	—
Trifolium pratense	4–6	18	10	6	2	trace	5	1	trace
T. hybridum		20		12	1		—	—	—
T. repens	—	—	—	trace	48	36	20	17¶	16
Miscellaneous herbs	—	5	7	4	12	7	8	10	10

* Figures = percentage productiveness.   † Figures = percentage frequency.
‡ *Dactylis* and *Phleum* not included in the mixture.
§ Mostly *Poa trivialis.*   ¶ Includes 3 % *Lotus corniculatus.*

TABLE VI.   *To show seeds mixture used and the contribution of the various species to the herbage on six fields at different ages, at 300'–550' above sea level.*

Contributing species	Seeds mixture in lbs. per acre						First year's hay	2½ years' pasture after one hay crop	2½ years' pasture after one hay crop	3 years' continued hay	2 years' pasture after 2 years' hay	10 years' pasture after 2 years' hay
	A	B	C	D	E	F	A*	B†	C†	D*	E†	F†
Age of fields							1	3½	3½	3	4	12
Lolium Italicum ...	—	4	—	3	—	3	—	—	—	—	—	—
L. perenne ...	—	10	—	—	12	12	—	11·9	6·4	—	7	5
Dactylis glomerata	8	3	8	14	6	3	14	1·3	9·5	40	9	6
Phleum pratense ...	2	2	3	—	2	2	4	1·6	4·9	—	2	2
Festuca elatior ...	6	—	—	3	—	2	1	—	—	—	—	—
F. pratensis ...	—	4	5	—	—	4	—	1 8	2·1	—	—	—
F. duriuscula‡ ...	—	1	1	—	} 2	1	—	2·1	4·3	1	8	3
F. rubra ...	—	—	—	—		—	—					
Alopecurus pratensis	3	2	3	—	—	2	—	·2	1·4	—	—	—
Poa trivialis ..	1	1	1	1	—	½	3	5·9	15·2	—	4	} 1
P. pratensis ...	—	—	—	—	1	½	—	—	—	—	7	
Arrhenatherum avenaceum	4	—	—	6	—	—	18	—	—	8	—	—
Trisetum flavescens	—	—	—	1	—	1	—	—	—	15	—	traces
Anthoxanthum odoratum	—	—	—	—	—	1	—	4·4	2·7	—	16	22
Cynosurus cristatus	2	—	—	—	2	—	11	3·3	1 2	—	9	13
Holcus lanatus ...	—	—	—	—	—	—	—	20·8	23·9	—	2	3
Agrostis spp. ...	—	—	—	—	—	1	—	22·8	5·8	—	13	13
Other grasses ...	—	—	—	—	—	—	1	3·9	2·3	1	—	—
Trifolium pratense	—	2	—	—	2	2	—	} ·5	1·2	6	1	2
T. pratense (late flowering)	—	—	3	2	—	1	—					
T. hybridum ...	5	1	2	1	1	2	26	—	—	1	—	—
T. repens§ ...	4	2	2	2	2	2	2	} 11 5	9·1	—	9	17
Medicago lupulina	2	2	2	—	—	—	12		—	—	—	—
Anthyllis Vulneraria	—	—	2	3	—	—	—	—	—	trace	—	—
Cichorium Intybus	—	—	1	3	—	—	—	—	trace	26	—	—
Achillea Millefolium	—	—	·5	—	—	—	—	—	2·7	trace	—	—
Poterium Sanguisorba	—	—	·5	8	—	—	—	—	—	trace	—	—
Miscellaneous herbs	—	—	—	—	—	—	7	8·0	7·3	2	13	13

* Figures = percentage productiveness.   † Figures = percentage frequency.
‡ The commercial seed.   § The commercial white or Dutch was sown.

The chief results obtained when complete mixtures were used on fields also receiving farmyard and phosphatic manures are set out in Table VI.

Some further observations may be briefly stated as follows:

Festuca duriuscula (commercial seed). 3 lbs. to the acre gave nothing in the first year's hay.

Dactylis glomerata. 2 lbs. to the acre gave 4% in first year's hay. 8 lbs. to the acre gave 12 % in first year's hay.

Arrhenatherum avenaceum. 2 lbs. to the acre gave 2 % in first year's hay.

Poa pratensis. 2 lbs. to the acre gave 2 % in first year's hay.

Poterium Sanguisorba. 6 lbs. to the acre completely covered the ground on a dry bank where everything else had failed, in the first year's hay crop.

These results are discussed in the summary at the end of the section.

C. Fields below 300' on Well-drained Alluvia or Gravel.

Some fifteen fields have been investigated upon this type. On the best soils, when farmyard and phosphatic manures are freely applied, a good indigenous herbage may be rapidly induced. This is well shown by the figures given in Table VII; where a field in its second year

Table VII. *To contrast the herbage on a field in its second year (one hay crop and one year pasture) with an adjoining permanent pasture at 100' above sea level on a rich soil.*

Contributing species	The two year old field	The permanent pasture
Lolium perenne ..	13	22
Anthoxanthum odoratum	—	9
Holcus lanatus... .	3*	2
Agrostis alba	4*	18
Dactylis glomerata .	—	2
Poa trivialis	22*	} 13
P. pratensis	24*	}
P. annua	4*	—
Festuca rubra	—	6
Cynosurus cristatus ...	—	4
Bromus mollis... ...	4*	1
Trifolium pratense ...	1	traces
T. repens	13*	13
Miscellaneous herbs ..	12*	10

The figures = percentage frequency.

* Have come in indigenously.

(sown with perennial rye grass 16 lbs. and red clover 12 lbs.) is contrasted with an adjoining old pasture (over 100 years down).

TABLE VIII. *To show seeds mixtures used and the contribution to the herbage of the several species on fields of different ages below 300'.*

Contributing species	Seeds mixture in lbs. per. acre				1 year's hay and 1 year's pasture		Hay every year		Pasture after 3 years' hay		8 years' pasture after 2 years' hay
					A*	B*	C†		C*		D*
	A	B	C	D							
Age of fields...					2	2	3	4	5	6	10
Lolium Italicum	4	5	2	—	3·0	1·5	—	—	—	—	—
L. perenne	—	—	20	20	19·6	30·6	14	8	8	10	12
Dactylis glomerata	8	—	2	—	5·2	—	15	19	3	2	—
Phleum pratense	—	—	1	—	—	—	22	15	3	1	—
Festuca pratensis	5	6	—	—	2·3	3·1	—	—	—	—	—
F. elatior	4	5	—	—			—	—	—	—	—
F. duriuscula	2	2½	—	—	0·5	0·2	—	—	—	—	—
F. rubra	—	—	—	—	—	1·2	?	?	4	2	9
Arrhenatherum avenaceum	3	3½	—	—	1·0	0·3	—	—	—	—	—
Trisetum flavescens	¼	¼	—	—	1·3	0·2	—	—	—	—	—
Poa trivialis	½	—	—	—	21·3	28·4	4‡	13‡	7‡	15‡	12
P. pratensis	2	2¼	—	—	0·5	2·9	—	—	—	—	
Holcus lanatus	—	—	—	—	14·4	11·7	20	12	10	9	7
Cynosurus cristatus	—	—	—	—	11·5	2·8	3	3	2	8	4
Agrostis alba	—	—	—	—	—	0·3	12	10	40	15	42
Other grasses	—	—	—	—	0·5	0·7	—	—	—	—	—
Trifolium pratense	2	2	1	8	0·1	0·2	2	2	1	3	—
T. pratense perenne	2	2	—	—			—	—	—	—	—
T. hybridum	2	2	1	—	—	trace	4	trace	—	—	—
T. repens (Dutch)	2	2	1	—	7·2	5·8	2	5	14	21	7
T. minus	—	—	—	—	0·9	2·2	—	—	—	—	—
Medicago lupulina	—	—	1	—	—	—	—	—	—	—	—
Anthyllis Vulneraria	2½	3	—	—	—	—	—	—	—	—	—
Achillea Millefolium	1	1⅛	—	—	0·8	0·6	—	—	—	—	—
Cichorium Intybus	2	2½	—	—	0·1	traces	—	—	—	—	—
Miscellaneous herbs	—	—	—	—	9·8	7·3	2	13	8	14	7

* Figures = percentage frequency.   † Figures = percentage productiveness.
‡ Including some *Poa pratensis*.

The rapidity with which *Poa* spp. and *Trifolium repens* have come in although not included in the seeds mixture is probably to some extent accounted for by the liberal dressing of basic slag (6 cwt. to the acre) applied to the young seeds after the covering crop (barley) was harvested—which produced a great development of red clover in the first year's hay and again in the aftermath. The red clover,

however, completely spent itself by the second year; but the soil so
enriched and still receiving the benefit from the basic slag was evidently
a most suitable habitat for the indigenous *Poa* spp. and *Trifolium
repens*, which had neither to compete with an excess of *Trifolium
pratense* or of *Lolium perenne* (which had been largely suppressed by
the great development of clover in the first year).   This case[1], although
probably somewhat exceptional, is very instructive and throws much
light on the whole question of pasture formation.

Detailed results obtained on six fields where more or less complete
mixtures had been used are given in Table VIII.   The figures under
columns A and B were obtained from the same field on two separate
plots of about three acres each.

The two mixtures used were similar except that in one case no
cocksfoot had been used; and in the other the seeding of the remaining
species had been increased to supply approximately an equal number
of germinating seeds in the two cases.

Some further results may be briefly stated thus:

A field twelve years down, sown with *Lolium perenne, Dactylis glome-
rata* (2 lbs.) and *Trifolium pratense* only, and which had yielded eight
crops of hay, gave

						per cent.
Leguminosae — *T. pratense* and *T. repens; T. minus*						
Lathyrus pratensis and *Vicia* spp.					...	25
Miscellaneous herbs (chiefly *Rhinanthus* spp.						
Centaurea nigra						
Rumex spp.					...	34
Plantago lanceolata						
and *Hypochaeris radicata*)						
Dactylis glomerata	...	...	...	...	...	8
Lolium perenne ...	...	...	...	...	...	8
Cynosurus cristatus	...	...	...	...	...	13
Poa trivialis ...	...	...	..	...	...	2
Holcus lanatus ...	...	...	...	...	...	4
Anthoxanthum odoratum ...	...	...	...	...	...	4
Other grasses ...	...	...	...	...	...	2

On Fields 8–10 years down the following species frequently contri-
bute up to

Poa trivialis	...	12 %	although not sown
Bromus mollis (et spp.)		17 %	„ „
Festuca rubra	...	10 %	„ „
Dactylis glomerata...		14 %	when sown

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[1] The case quoted is typical of all the fields on the farm of which it formed a part;
the farmer, a most skilled cultivator, holding the view "Do everything to obtain a great
development of Red Clover in the first year and the herbage will subsequently look
after itself."

On one eight-year field used as a meadow in alternate years, *Dactylis glomerata* contributed 30 % to the herbage.

SUMMARY OF CONCLUSIONS TO BE DRAWN FROM THE FOREGOING TABLES AND STATEMENTS AND FROM OUR INVESTIGATIONS GENERALLY BEARING ON THIS SECTION.

It will be convenient to discuss the behaviour of the chief plants found on all the types of grassland investigated under a separate heading for each individual species.

GRASSES AND CLOVERS.

(a) *Primary species.*

Agrostis vulgaris. Although a primary species it may come in early in the life of a field both below and above 600'; when plentiful in the first or second year it is, however, usually due to a poor "take" and a "foul" seed bed. Meadow conditions tend to suppress and pasture conditions to favour its development (see Tables II, III and IV). Under 10–20 years' pasture it usually attains to a higher percentage (*e.g.* 20 %–60 %) than it does on unbroken heath pastures (*e.g.* 7 %–25 %). With proper manuring and attention this need not. however, be the case, for by this means *Cynosurus cristatus* and *Poa* spp. can be brought into strong competition with it. A large sowing of cocksfoot (8–14 lbs.) would appear also to suppress the development of this grass.

Agrostis alba. This primary species on well-drained alluvia is not usually plentiful until the third or fourth year and thenceforward tends to gain rapidly. It is favoured by grazing, but is a much more successful meadow plant (on good soils and at low elevations) than is *A. vulgaris* under any conditions.

Festuca spp. (fine leaved). The primary species above 600' is *F. ovina*, with *F. rubra* secondary indigenous. At low elevations and on good soils *F. rubra* is to be regarded as a primary species. *F. ovina* is invariably late to come in (except on reclaimed fields immediately reverting to grass), it seldom contributes much to the herbage before the seventh or eighth year (see Table II) and even by the twentieth will not have attained to the same predominance which it reaches on the natural fescue pastures (20 % is a high figure on fields twenty years down; 20 %–45 % is common on the natural pastures).

F. rubra may come in naturally as a secondary species in the third year below 600' and as a considerable contributor by the fifth year

above 600' (see Tables III and IV). It also comes in early (in small amount) on good soils at low elevations and lasts for over a hundred years which suggests its being a primary species under these conditions (see Tables VII and VIII). It would appear that both *F. ovina* and *F. rubra* are favoured by pasture conditions.

It is difficult to estimate with certainty the effect of sowing the various commercial counterparts of the indigenous fine leaved fescues. The so-called *F. duriuscula* of commerce is frequently sown in Wales and on the Cotswolds. On the Cotswolds one of us was able to prove that the inclusion of this seed (4 lbs. to acre) gave only 3·2 % fine leaved fescues to the herbage in the second year and the majority of this was demonstrably the indigenous *F. rubra* var.

In Wales where both 1 lb. and 2 lbs. of *F. duriuscula* (?) had been sown, the contribution of fine leaved fescues to the herbage was no greater than that reached on other fields by the unsown indigenous *F. rubra*; and on no pasture of any age have we found more than 3 % of *F. duriuscula*[1]. At high elevations we have found no benefit from including commercial *F. ovina* in the mixtures. The available evidence is therefore all against including the commercial fine leaved fescues in seeds mixtures in the districts under review.

Molinia caerulea, Nardus stricta, and *Triodia decumbens* are primary species very susceptible to interference and do not attain to a prominent position, at all quickly, after a field has been properly reclaimed. *Triodia decumbens* seldom appears before the seventh year but may be plentiful by the eleventh. *Molinia* and *Nardus* only appear before the tenth year if fields are quite neglected.

(b)   Secondary species (a few are possibly primary on good soils).

Lolium perenne behaves as a primary species on good soils; but since it is always included in mixtures it is difficult to say how rapidly it comes in indigenously on suitable habitats. Considerable quantities have been met with in the second year on small plots (see B, Table VIII) where it was excluded from the mixture; but this may well have been due to seeding from adjoining plots. It frequently attains to a high percentage up to the third or fourth year from sowing, then drops for a few years, and subsequently rises. This suggests that the plants met with after 12–20 years on a good soil are the indigenous counterparts of the original sowing. Above 600' it is probably a local exotic[2], for

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[1] Or of any fine leaved fescue apparently different to the locally indigenous varieties.
[2] See footnote 1, p. 55.

it falls off very rapidly from sowing and only persists under abundant manuring. It is better under pasture than meadow conditions (see Tables II, III and IV). On all situations it is encouraged, relatively to other grasses, by being trampled by cattle in winter. On poor soils below 600' it succeeds better than at higher elevations and possibly occurs as a secondary plant[1].

Poa trivialis behaves as a primary plant on good soils, and, since it occurs on such situations as an arable land weed, it may make an early and abundant indigenous appearance (see Table VII). Its appearance is hastened by a great development of clovers in the first year and by liberal manuring. On the majority of ordinarily good soils, however, the commercial seed included in a mixture both makes its appearance more certain and hastens its development.

On poor soils above 600' it appears only to be a slight secondary plant– but we have seen fields so situated where it has succeeded well from seeding under liberal manuring. On poor soils below 600' it is a secondary plant of surprisingly general distribution (see *e.g.* Table V) and when reliable seed is used, it may add appreciably to even the first year's hay (1 lb. to acre having given 2 % to the hay crop). The figures given in both Tables V and VI suggest that it is favoured by pasture conditions. The success of this plant on thin soils is noteworthy and is to be attributed to high rain fall and a moist atmosphere. This plant deserves close attention for clever management may often render its inclusion in mixtures unnecessary.

Poa pratensis is probably a slight secondary plant both above and below 600'; but is only really prominent as a secondary plant on good soils. The available evidence seems to show that even from seeding it is not so successful as *P. trivialis*.

Anthoxanthum odoratum is a slight primary and fairly abundant secondary plant on the majority of soils, but does not usually come in in any quantity until the third or fourth year; but at lower elevations may then increase rapidly.

Cynosurus cristatus is a secondary indigenous plant on all the types investigated; but at high elevations and on poor soils does not come in to any extent naturally until the third or fourth year and only slowly (*e.g.* after 8–10 years) reaches its maximum development.

On good soils at low elevations it may come in earlier (the very high figure under A, Table VIII, is probably largely due to seeding from an

[1] It is always included in mixtures and the plot evidence available is not sufficiently reliable.

adjoining plot) but it usually works only slowly up to its maximum contribution. On the average of all the figures obtained it would seem to succeed as well under meadow as pasture conditions. Although this plant will certainly come in naturally, it seems advantageous to include it in mixtures especially on the poorer soils. The commercial seed ensures a quicker development and will guarantee an appreciable contribution to the first year's hay, 2 lbs. at 600′ having given 5 % of the total hay.

Our observations and analyses do not agree with those of Carruthers [4] with respect to this grass. We have found, when making our separations, that the green herbage always shows evidence of having been grazed by stock and we have also had evidence of differential grazing in favour of this plant. On poor soils in the districts under review it is an extremely valuable grass for both pastures and meadows.

Trifolium repens is a strong primary plant on the better soils and a no less strong secondary plant on poorer soils when phosphatic manures are freely employed.

As an indigenous plant it does not usually come in very strongly until the third year and then gains rapidly; usually more rapidly under pasture than meadow conditions.

Evidence to show that any advantage is to be gained by including the ordinary and expensive commercial seed in mixtures is very slight; Table II (no seeding) can show as good results as Table III (seeding). Table V without seeding shows actually better results from three to eight years than does Table VI with seeding. Two lbs. included in the mixture (Table VIII) seems, however, to have given in the first year better results than 1 lb. in the mixture.

Trials are now being conducted with wild white clover in both North and Mid Wales; plots in Flintshire already show that the wild white has given better results in the first year than the ordinary commercial seed. It would seem probable, from what has been said above, that if phosphatic manures do not "bring" the indigenous plant, the ordinary seed will not always materially assist matters, but that small seedings of wild white are then essential[1].

Trifolium minus is a strong secondary plant on poor soils, but tends to come in rather late; and, since much of the commercial seed consists of a high percentage of "hard" seed, it is not probable that sowing can be relied upon to materially hasten its appearance.

[1] Cf. Gilchrist (10)

Trifolium pratense from seeding is not a lasting plant. Plot experiments have shown Welsh and English grown seed to give more lasting plants than imported stocks. The secondary indigenous plant comes in naturally at about the eighth year.

Vicia Cracca. A fairly common secondary indigenous plant on poor soils at high elevations, appearing rapidly on fields recently reclaimed, and, in the fifth or sixth year, on fields long under the rotation.

Lotus corniculatus. A secondary plant to come in early on land recently reclaimed, and not to any extent before the third year on land long under the rotation. We have once seen good results from the use of commercial seed in the first year.

(c) *Species locally secondary indigenous, locally exotic or exotic*[1].

Dactylis glomerata. All the evidence shows that, whereas this grass is often abundant as an indigenous plant in waste places and in light woods or plantations, on hedges and by roadsides, it seldom appears as a secondary plant on tended grasslands in these districts[2] (*i.e.* it is usually a local exotic). The figures in the tables further show that 4 lbs. and less sown to the acre have given negligible results under pasture conditions (see Table III and Table IV B); whilst under hay (or hay for the greater number of years) even such small sowings have given substantial results from the second to the fifth year (Table IV), in the first and fourth years (Table V), in the third and fourth years (Table VIII), and a field has been cited where 2 lbs. *Dactylis* under eight years' continued hay gave 8 % to the hay in the eighth year. When, however, this grass is sown in large amount 6-8-14 lbs., it frequently contributes substantially to the first year's hay (Table VI A), may dominate the third year's crop (Table VI D) and contribute materially to the subsequent pastures (Table VI C and E). From the above it would seem (a) that *Dactylis glomerata* cannot readily gain on the ground from a

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[1] The term "locally secondary indigenous" is applied to species which are not normally secondary plants on a type, but which occasionally appear in small amount only. "Locally exotic" is applied to plants which are indigenous species in a district, *e.g.* in waste places, hedgerows, coppices, etc., but which do not occur on well-marked natural or semi-natural grasslands. "Exotic" implies a plant which is not indigenous on any habitat in the district (although in the case of nearly all the plants thus designated in this paper a member of the British Flora).

[2] On many of the fields (but by no means the majority) investigated by Carruthers (4) and (5) *Dactylis glomerata* would seem to have been a secondary plant; so that it probably does so occur on some types of grassland; especially when meadow conditions largely obtain.

small seeding; hence a few seeds fortuitously introduced from a hedge or elsewhere are without significance. (*b*) If allowed to run to hay, even from a slight original sowing (the hay is always cut too late in these districts) it rapidly gains on the ground by virtue of self seeding. (*c*) As Carruthers(4) has shown, this grass is undoubtedly favoured by meadow conditions. (*d*) Being a strongly compacted caespitose grass, it cannot gain on the ground under pasture conditions, but if sown in large amount it can be made to serve as one of our most valuable pasture grasses.

Phleum pratense. This is possibly a secondary plant on some of the better soils; but there is no evidence that it is so on the poorer ones, where it is probably locally exotic or exotic.

Many farmers aver, however, that if once sown on a field, it will always afterwards re-appear when that field is under grass. It also occurs as a common impurity in *Trifolium hybridum,* and this would frequently account for its " unsown " entry into a field. It often succeeds well on poor soils, presumably owing to high rain fall; it succeeds best under meadow conditions, but is somewhat fickle and requires further accurate study.

Arrhenatherum avenaceum[1]. Like *Dactylis glomerata* although common about hedges and elsewhere, it seldom occurs as a secondary plant to any extent on tended grasslands (on most types it is locally exotic). When sown it succeeds best under meadow conditions.

Trisetum flavescens[1] is possibly a rare and local secondary plant on some types; has sometimes shown itself able to succeed from seeding (*e.g.* Table VI D).

Festuca pratensis[1] and *F. elatior*[1]; the former plant is at best a rare secondary plant on some of the more fertile types, but is elsewhere locally exotic; the latter is locally exotic or exotic on all the types under review. *F. pratensis* is probably never an economic success above 600', although a few plants have been found on fields so situated five years after sowing, and even on the better soils at low elevations good results are the exception. *F. elatior* has proved more successful at high elevation, but is uncertain and does not seem to offer advantages which are not also possessed by the more reliable *Dactylis glomerata*.

Alopecurus pratensis[1] is locally exotic or completely exotic to the types on the poorer soils at high elevations and is only a local secondary species on the more fertile soils at low elevations. An indigenous plant

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[1] Locally purchased seed is too unreliable in the case of these plants to base much value on the evidence given when such seed is included in the mixtures.

is frequently seen about waste land near towns, but we have not at present experimented with seed collected from it. It has sometimes proved successful from commercial seeding on fertile soils especially when farmyard manure is freely used.

Holcus lanatus. This is an indigenous plant on many types of grass-land; it is presumably a primary plant on the Bridgwater flats in Somersetshire and elsewhere, and it is probably a slight primary plant on some of the more ill-drained types in the districts under review. On poor soils up to the extreme limits of cultivation it rapidly gains on fields especially under meadow conditions, usually reaching its zenith at the third or fourth year and then falling slightly even if hay is still taken (Table II); but it decreases very considerably under grazing. On better soils (or at all events wetter ones) it is still favoured by taking hay, but is more tolerant of constant grazing than on the poorer soils (*cf.* Table II with Table VIII C and D). It is certainly not a long lived perennial on the poorer soils above about 400', where it gains on the ground by abundant self seeding; and since it is a universal impurity in the rye grasses[1] commonly sown in these districts and can also be carried freely from field to field by the wind, the evidence is against its being even a secondary plant on the types investigated on soils derived from Terannon shales. On the alluvial soils, since it may there long continue under constant grazing, it is probably a secondary or primary plant. Very large sowings of *Dactylis glomerata* (10–14 lbs.) would seem to have a decidedly depressing influence on *Holcus lanatus* even under meadow conditions (see Table VI D). At the College Farm, Aberystwyth on permanent manurial plots for hay (on a field over eight years down) the average percentage yield of *Dactylis* is 35 %, of *Holcus* only 4 %.

Bromus mollis et spp. is not so abundant as *Holcus* on the types investigated, but is probably a truly secondary plant on alluvia and is a slight secondary plant on the poorer soils below 600'; it may become abundant when the seed is introduced as an impurity. Is essentially a plant of meadow conditions.

Trifolium hybridum. This exotic, on some soils yields as well in the first hay crop as *T. pratense*; but falls off rapidly after the second

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[1] 91 % of the samples of perennial rye grass and 78 % of the Italian rye grass samples tested at Aberystwyth in 1913 contained Yorkshire Fog in appreciable amount. Sweepings from the hay loft are still frequently sown. The farmers of the last generation were even more careless than those of the present in their purchase of seed; consequently the abundance of Fog on certain types of grassland is probably the heritage of negligent farming.

year, although we have occasionally seen plants on a field even up to the twelfth year.

Anthyllis Vulneraria. Although a primary plant on some types near the sea, is locally exotic to all the types under review. Sometimes gives good results in the first hay crop on soils derived from Terannon shales, but is not persistent and has only shown traces in the third year (Table VI D).

Medicago lupulina is a secondary plant on limestone soils in Pembrokeshire and North Wales, but is exotic on all the types here discussed. It is largely sown in these districts, but above 600' is a complete failure, seldom even contributing to the first year's hay; below 600' it sometimes bulks satisfactorily in the first hay crop but does not appear to last into the second year.

Medicago sativa is an exotic plant which we have never seen succeed in mixtures on any of our types; on gravel it sometimes contributes slightly to the first year's hay crop.

Miscellaneous herbs.

The competitive interaction of miscellaneous plants between themselves and with the grasses and clovers has been dealt with elsewhere by one of us in so far as the first year is concerned[1] and is still under investigation with regard to the later years, consequently but few species will be here discussed.

Species which are frequently sown.

Achillea Millefolium is probably a slight secondary plant on all the types reviewed; but when unsown is usually late (about sixth or seventh year) to come in naturally in appreciable amount (Table III). Seeding would seem to hasten its appearance.

Cichorium Intybus is exotic on all the types, but sometimes makes a sporadic appearance being a sown impurity with foreign red clovers. From large seedings it often succeeds well on the soils derived from Terannon shales and has shown itself able to persist until the third year (Table VI D) and in traces until the fourth year.

Poterium Sanguisorba. Exotic to our types and not a general success when sown; but sometimes completely dominates the ground on dry banks.

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[1] See Stapledon (18). In this connection it will be necessary also to recognise Tertiary Species, *e.g.* such as can thrive under grassland conditions for a few years and then completely disappear

Plantago lanceolata[1] is probably a secondary plant on the majority of the types; it attains to a more dominant position on old than young leys.

Species which are not advisedly sown.

Rhinanthus spp. This is a secondary plant on all types. It gains rapidly on the land under meadow conditions, especially in these districts where the hay is habitually cut late, usually reaching its zenith at about the fourth or sixth year; under subsequent grazing for three to five years it is greatly decreased. Strong competition with *Dactylis glomerata* does not appreciably decrease it, although giving it a relatively lower percentage productiveness.

Ranunculus repens. A dominant secondary plant on all types, being equally abundant as an arable weed it may be excessive even in the first year; but from a clean seed bed usually only attains to dominance in the later years of a ley, but may attain to its maximum figure by the sixth year. Evidence is not lacking to suggest that *Trifolium repens* competes favourably against it[2].

Bellis perennis, Prunella vulgaris, and *Sagina procumbens* are puzzling and interesting plants concerning which we are at present unable to generalise usefully.

Potentilla erecta and *Galium saxatile* are primary plants on all semi-natural types derived from heath, which seldom reappear on tended land before the fifth or sixth, and frequently not until the tenth or fifteenth year.

VI. SUMMARY CONCLUSIONS.

(1) Grassland has been classified as natural and semi-natural; both classes have been further sub-divided according to the botanical composition of the herbage in relation to habitat (pp. 27–36).

(2) The plants which colonise natural grasslands have been called primary indigenous species; and those which come in without being sown and contribute largely to the herbage on semi-natural types have been designated secondary indigenous species (p. 37).

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[1] Since the plant is still largely sown above 600′ and used to be sown to an even greater extent, and is still abundant as an impurity in clovers, it may well be, like *Holcus lanatus*, a heritage of the husbandry practised on the types above 600′ and not a secondary plant.

[2] *Ranunculus bulbosus,* the chief Ranunculus on the Cotswolds where it is not an arable weed, invariably makes a late appearance on leys.

(3) Plants which come in by themselves on young leys but which disappear as the field approaches to the semi-natural may further be called Tertiary indigenous species; these have been discussed provisionally elsewhere (see footnote 1, p. 58) and will be dealt with in detail in a subsequent paper. Plants which are indigenous in a district but which do not naturally contribute to the flora of a well-marked type of grassland have been called locally exotic; and those which are not indigenous in a district exotic (see footnote 1, p. 55).

(4) The above classifications and distinctions are applicable to all districts and to all types of grasslands. It must, however, be emphasised that what has been brought forward concerning every individual species is only claimed to apply *in toto* to the types of grassland investigated and in the districts under review. A knowledge of other counties, however, suggests that many species would fall into the same categories and behave in a similar manner elsewhere. The following generalisations are, at all events, justified by the data here produced.

(5) The number of primary species on most types is not considerable. When a field long under rotation husbandry is put down to grass, the primary species are usually late to come in; this is particularly true of *Bromus erectus* (on the Cotswolds), *Molinia caerulea, Nardus stricta, Triodia decumbens,* and *Festuca ovina*; and if they come in early, they do not rapidly make their normal contribution to the herbage[1]. Primary species which sometimes come in early are plants which are frequently met with as arable land weeds, *e.g. Poa trivialis,* and *Festuca rubra* (at lower elevations and on the better soils) and *Agrostis vulgaris* (on the poorer soils).

(6) The various stages in the process of stabilisation of semi-natural grasslands —through tended to untended and thence into natural types—have been summarised at the end of Section IV (pp. 39–40).

(7) The relation of primary and secondary species to their commercial and sown counterparts is as follows (see also summary to Section V, pp. 51–59). Either

(a) The commercial seed does not appreciably hasten the appearance or add to the contribution of the desired plant. This is true of sowing *Festuca ovina,* or other fine leaved fescues and is, in many cases, equally true of *Anthoxanthum odoratum*; the inclusion of such seeds in mixtures is not justified economically. It is far from certain that

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[1] *I.e.* they may fall below their minimum or exceed their maximum figures. *Poa trivialis* for instance on good land often exceeds its maximum figure on young leys.

the commercial *Trifolium repens* (white or Dutch clover) produces a lasting plant, on many types phosphatic manures being all that is necessary to hasten the appearance of the indigenous plant.

(b)   The commercial seed may produce a great bulk of the required plant in the early years of a ley (far more than the indigenous species would attain to naturally at any time).   It is only after some years that the plant attains to its normal development, which suggests that the final plant is the indigenous counterpart of the sown species. The behaviour of *Lolium perenne* sown on good soils is an excellent example.

Under these circumstances good seedings of the commercial seed is economically justified especially if hay is required in the early years of the ley.

(c)   The commercial seed may hasten the appearance of the desired plant and cause it to bulk somewhat more largely in the early years of a ley than it otherwise would; but there is always some risk of the sown plant interfering with the development of the definitely lasting indigenous species.   Good examples are *Poa trivialis,* and *Cynosurus cristatus*; ultra local knowledge would often suggest excluding the former from a mixture; while the amount of seed of either that might be advantageously used requires further local investigation.

(8)   The desirability or otherwise of sowing the commercial seed of locally exotic species, or of but slightly secondary species, is easily decided.   The commercial seeds lead to good results or they do not. If the commercial seed produces plants that are found to succeed it is however nearly always necessary to sow liberally; this is particularly true of *Dactylis glomerata, Festuca elatior, Cichorium Intybus, Phleum pratense,* and *Arrhenatherum avenaceum.*

(9)   It is difficult to account for the spontaneous appearance of primary and secondary species (and of some locally exotic species, *e.g. Holcus lanatus, Bromus mollis* et spp. and *Phleum pratense*) on land long under the rotation when put down to grass; especially when more or less isolated from natural or semi-natural grasslands.   The available evidence, however, suggests that

(a)   Many species remain on the land as arable weeds, *e.g.* on the soils that suit them, *Poa trivialis, Agrostis vulgaris, ·Festuca rubra,* and *Ranunculus repens.*

(b)   The seeds of many species are probably introduced by the wind *e.g. Holcus lanatus, Anthoxanthum odoratum.*

(*c*) The seeds of many species are certainly introduced as impurities (useful and otherwise) in the sown seeds, *e g.* *Phleum pratense* (in *Trifolium hybridum*), *Plantago lanceolata* (in *Trifolium* spp.), *Holcus lanatus* and *Bromus mollis* et spp. (in *Lolium* spp.).

(*d*) There seems little doubt, however, that the seeds of a great number of species are capable of lying dormant for long periods in the soil; in particular we suspect this to be true of *Trifolium repens* and *T. minus, Cynosurus cristatus, Poa* spp., *Phleum pratense,* and *Festuca ovina.*

The data brought forward in this paper would seem further to justify the following broad generalisations with regard to both experimental work on grassland and the whole problem and economics of putting land down to grass

(1) Experimental plots dealing with seeds mixtures should be large (at least half an acre) and square in order to give a considerable central zone. The hay should be cut as early as possible to avoid seeding and the carriage of seed from plot to plot. No series of plots can, henceforward, be regarded as complete without a control plot, which control should not be seeded (with grasses and clovers) but left to the indigenous species to colonise. The control plot should, of course, be subjected to the same cultivations, receive the same manures, and grow the same nurse as the seeded plots.

(2) Undoubtedly when putting land down to long duration grass as much or more can be done by making the habitat as suitable as possible to the desirable indigenous species as by including their commercial counterparts in the mixture. The commercial permanent grasses are far more valuable for say 4–6 year leys than they are for permanent grass as such

Poa spp. and *Cynosurus cristatus* to some extent tide a field over its critical third and fourth years in proportion to the sowing, but in the later years the amount of the original seeding becomes of small significance compared to the influence of proper manuring and general management. Speaking generally pasture conditions favour the valuable indigenous species better than meadow conditions. On poor soils especially we are accumulating evidence to show that rape[1] (folded on the land) is a much better nurse than oats or barley (removed from the field).

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[1] Mr Wibberley also informs us that he has got excellent results under rape on mountain land in Ireland.

(3) Our indigenous herbage plants offer a promising field for study. Nor should modern investigators confine their attention to grasses and clovers only, with the exception of but few miscellaneous herbs. We have been struck in the course of our work by the extent to which such plants as *Juncus squarrosus, J. Gerardi, J. articulatus, Bellis perennis,* and *Statice maritima,* are relished by stock.

It is, however, most desirable to study the locally successful varieties of *Festuca ovina, Festuca rubra* (with the other fine leaved fescues), *Poa trivialis, Poa pratensis, Lolium perenne,* and *Cynosurus cristatus,* with a view to estimating their relationship to the commercial counterparts and if necessary with a view to establishing local supplies of the indigenous seed. To do, in short, for these species what has been so successfully done in the case of wild white clover as the result of the investigations of Gilchrist (10) and others.

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THE REACTION BETWEEN DILUTE ACIDS AND THE PHOSPHORUS COMPOUNDS OF THE SOIL.

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FEW reactions are more important to the soil chemist than that involved in the action of dilute acids on the phosphorus compounds of the soil, but, owing to its complex nature, little has been definitely ascertained about it. The importance of the reaction lies in the fact that it affords a distinction between those phosphorus compounds which are fairly easily soluble, and may therefore be expected to enter the plant root without much difficulty, and the less soluble compounds which are of less value in the nutrition of plants.

The necessity for such a distinction was emphasised in a classical memoir published in 1845 by Daubeny[1], who used the terms "active" and "dormant" to express the more and less soluble constituents respectively. He suggested that a solution of carbonic acid might be used to discriminate between them, but the manipulative difficulties proved considerable, and the suggestion was gradually forgotten, and along with it the distinction it was intended to emphasise. It was not till 1894 that general attention was once more directed to the need for the distinction by the publication of Dyer's important paper[2] in which he uses the terms "available" and "unavailable" for these groups,— terms which have since been generally adopted in this country. From the circumstance that the earlier analyses were expressed not only in percentages but also in pounds per acre, the idea gradually arose that the "available" and "unavailable" compounds were sharply distinct.

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[1] Daubeny, C. G. B., "On the rotation of crops and on the quantity of Inorganic Matters abstracted from the soil by various plants under different circumstances." *Phil. Trans.* 1845, 179–253.

[2] Dyer, B., "On the analytical determination of probable available mineral plant food in soils." *Trans. Chem. Soc.* 1894, 65, 115–67; also *Phil. Trans.* 1901, 194B, 235–90.

Hall and Plymen[1], however, argued from their numerous analyses that there was no evidence of two definite groups, but that all the facts could be explained on the view that a considerable number of compounds occur of different degrees of solubility which, however, merge gradually one into the other.

Dyer used 1 % citric acid as a solvent, and found that it brought out amounts of phosphorus comparable with those which might be expected from a knowledge of the crop producing properties of the soil. This particular solvent had already been recommended by Tollens[2], and used by Stutzer[3] for the examination of phosphatic manures, but it was finally selected by Dyer on theoretical grounds. At that time plant roots were supposed to excrete acids that dissolved the soil phosphates and other mineral substances which then passed into the roots by osmosis. Now Dyer found that 1 % citric acid had approximately the same degree of acidity as cell sap, and argued that it must therefore exert approximately the same solvent action on the soil phosphates as the plant excretions, and would therefore give the most faithful picture of the phosphatic material available for plant nutrition.

The conception of the plant root as a special excreting and dissolving agent is now generally abandoned, as no satisfactory evidence can be obtained that any acid other than carbonic acid is excreted, or that any action beyond respiration is concerned. With this conception the theoretical basis underlying the selection of 1 % citric acid has gone too, and the method becomes purely empirical, and justifiable only by the extent to which its results are of value in soil analysis.

Judged by this empirical standard 1 % citric acid has proved fully satisfactory, and in Great Britain it is very generally adopted. Wood[4] and also Hall and Plymen[5] found that it gave results which accorded with the agricultural history of the soil. But it has not found acceptance elsewhere. N/200 hydrochloric acid has been recommended in the United States, and 2 % HCl (about N/1·82) in Sweden. Mitscherlich[6] adopts a saturated solution of carbonic acid, again, in order to simulate the action of the plant roots, and has carried out extended observations

[1] *Trans. Chem. Soc.* 1902, **81**, 117–44.

[2] A. Grupe and B. Tollens, *Ber. d. deutsch. Chem. Gesell* 1880, **13**, 1267; v Ollech and Tollens, *Journ. f. Landw.* 1882, **30**, 519.

[3] *Chem. Ind.* 1884, **7**, 37.

[4] Wood, T. B., *Trans Chem. Soc.* 1896, **69**, 287, also Wood and Berry, This *Journal*, 1905, **1**, 114–21.

[5] *Trans. Chem. Soc.* 1902, **81**, 117–44.

[6] *Landw. Jahrb.* 1907, **36**, 309–369.

to show that it gives results in agreement with those of pot experiments. Aspartic acid, acetic acid, and others have also been used.

From the fact that so many dilute solvents remain in use by analysts, it may be inferred that almost any acid can be made to give satisfactory results provided sufficient trouble is taken to ascertain suitable conditions of extraction. Direct experimental verification of this view was obtained by Hall and Plymen, who found that all the dilute acids gave the same kind of results, although there were considerable differences in the amount of phosphoric acid brought out; but citric acid proved to be on the whole at least as convenient as any other, and, as it had already been in use for some years, there was no advantage in giving it up. It is on this basis that the analytical side of the problem has solved itself, and in any country where considerable experience has been gained with a particular solvent there is probably little to be said in favour of making any change. The extensive literature that has grown up round this branch of soil analysis is largely concerned with the accumulation and record of experience of the particular solvent adopted, and need not therefore be discussed by us at this stage.

There is, however, a wider problem of more fundamental significance. By studying the reaction under definite conditions in the light of the well established laws of chemical dynamics it ought to be possible to discover the type of the reaction, and thus to obtain information as to the nature of the phosphorus compounds in the soil. The first attempts in this direction were made by Hall and Amos[1]. Soil was extracted with successive doses of citric acid and the P_2O_5 determined: the results were plotted in the usual way. The curves, however, could not be fitted by any of the ordinary equations. Similar negative results were obtained by de Sigmund[2] in an interesting series of investigations with nitric acid. Our own experiments also yield the same results. It may be taken as established that the reaction is not of the simple type presented by the familiar mono-, di-, or tri-molecular reactions of the text-books.

Hall and Amos considered that their results could be explained on the supposition that soil contains several phosphorus compounds of varying degrees of solubility. This may be so, but it cannot be the only factor. We shall show that citric acid extracts considerably more phosphorus from the soil than equivalent concentrations of nitric acid,

[1] *Trans. Chem. Soc.* 1906, **89**, 205–22.

[2] *J. Amer. Chem. Soc.* 1907, **29**, 929–36.

a fact which indicates that something more is concerned than a mere mixture of phosphates. One of us has shown, also, that Hall and Amos's results are better explained on the view developed in this paper[1].

Schloesing[2] states that the amount of phosphorus dissolved from soils is constant whatever the concentration of the acid between certain limits and de Sigmund[3] supported this claim. As his paper is not generally accessible to agricultural chemists his curve is reproduced here (Fig. 1). With very dilute acids the action is only slight: as the concentration increases more phosphate comes into solution. Then for a period the action is constant whatever the strength of the acid; finally, with stronger acid the action again increases, and goes on increasing with each addition to the strength of the acid. These results, if correct, would afford strong indication of the presence of an easily soluble phosphate which was being dissolved out during the period of constant action (the horizontal part of the curve), and of less soluble phosphates, which are only brought out by the stronger acids.

Initial acidity equivalents of HNO_3 per litre

de Sigmund's curve   Our results
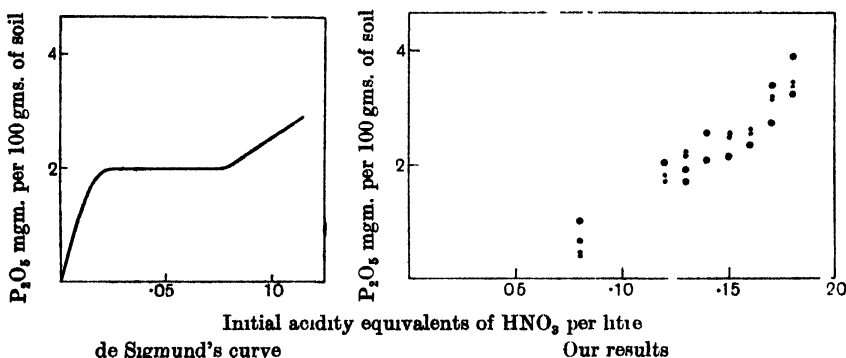
Fig. 1.   Amounts of P_2O_5 extracted from soil by HNO_3 of varying concentration.
Laboratory temperature.

We have carefully repeated these experiments but failed to obtain the same results. Both Schloesing and de Sigmund seem to have carried out their extractions at laboratory temperature, which in practice is somewhat variable. The improved method now available for estimating phosphoric oxide in solution enables us to plot the results on a larger scale than was possible for the earlier investigators. Working under their conditions the results are very variable, and fit neither a curve nor a straight line (Fig. 1, Table II), although if one reduced the scale

[1] J. A. Prescott, *Proc. Chem. Soc.* 1914, **30**, 137–8

[2] *Compt. Rend.* **128**, 1004.

[3] *J. Amer. Chem. Soc.* 1907, **29**, 929–36.

of plotting they might be made to fit a curve of the Schloesing and de Sigmund type, or, for that matter, almost any other. Arrangements were therefore made for shaking at constant temperature. Under these conditions uniform results are obtained, but the figures show no constancy over any period (Fig. 2, Table III). Thus the evidence of constant action disappears, and with it the evidence for the view that the action of dilute acids on soil is a simple solvent action, an easily soluble phosphate being first attacked, and then more difficultly soluble phosphates. We have just seen also that the action is not of the ordinary mono-, di-, or tri-molecular type.

The significance of these conclusions is very considerable. The soil was for a long time regarded as a collection of insoluble inert mineral fragments admixed with small quantities of more soluble substances some of which arose by weathering or other decomposition processes. This view had the advantage of simplicity, and it allowed of the application of simple and chemical and physical laws to soil processes. But unfortunately it leads to inaccurate conclusions. If soil is simply a mixture of insoluble inert minerals with phosphates, etc., there is no reason why it should behave abnormally towards dilute acids. In like manner the view leads to wrong conclusions as to the phenomena of evaporation of water from the soil[1].

It thus appears that this simple conception of the constitution of the soil is inaccurate, and must be discarded. We have been able to show that another conception is more in accordance with the facts.

The most convenient method of studying the reaction between dilute acids and the phosphorus compounds of the soil is to shake a definite weight of the soil,- we used 50 or 100 gms., with a uniform volume, —1 litre in our experiments, of the acid at constant temperature (23° C.) for a definite time, and then to estimate the amount of phosphorus compounds in the solution. Thus all the factors are under control and can be varied one at a time, all the others remaining constant. The results obtained are briefly summarised below.

1. When a soil is shaken for a definite period at constant temperature with a dilute acid the amount of action is found to increase continuously with the concentration of the acid. The increase is nearly proportional to the concentration of the acid, but not quite, and on plotting the results they are seen to fall on perfectly smooth curves

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[1] This *Journal*, 1914, **6**, 456–475; also *Annual Report of the Rothamsted Experimental Station*, 1914, p. 6.

Agdell Soil.
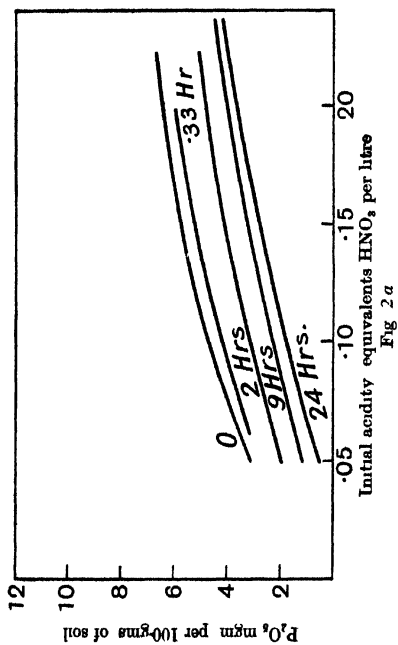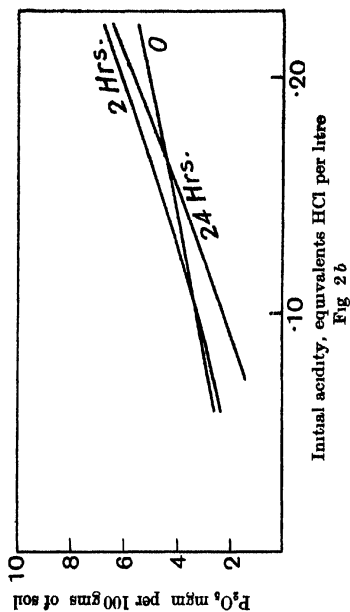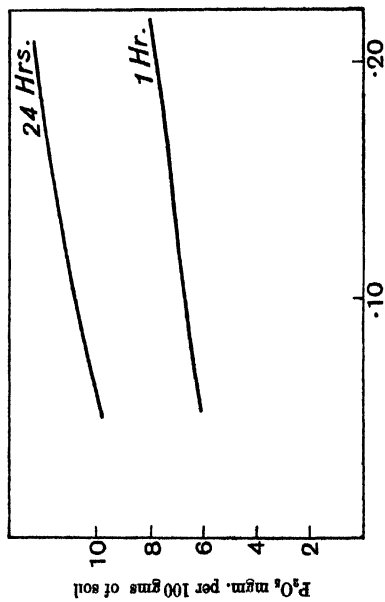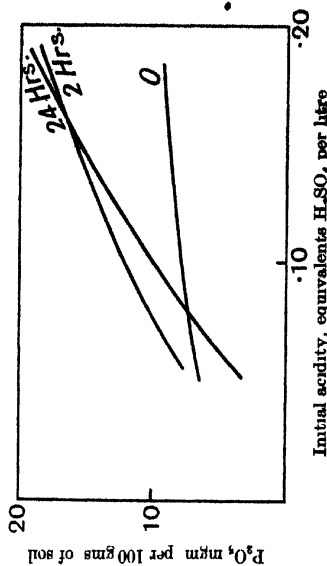
Fig 2　Amounts of P_2O_5 extracted from soils by acids of varying concentration acting for different periods of time at constant temperature (23° C)

which though very flat do not appear over any part of their course to be straight lines. There is no sign of any break in the curves, and nothing to indicate any definite stages in the reaction (Fig. 2, Table III).

Hoos 2 C Soil
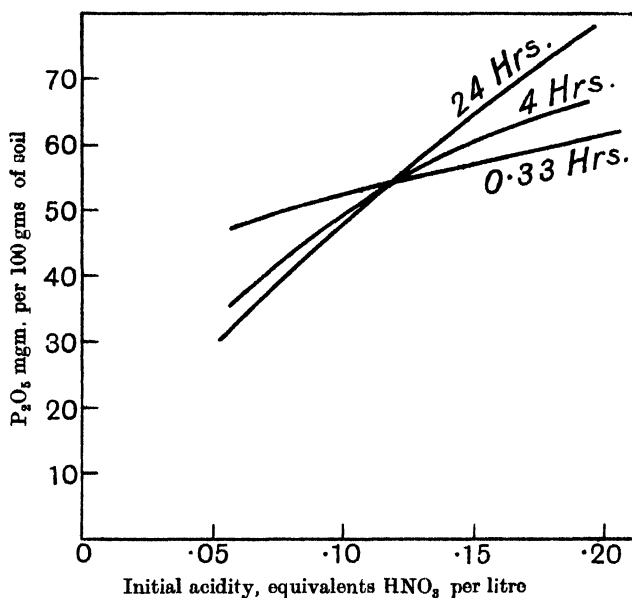
Fig. 2 e.

2. All dilute acids, as far as they have been examined, give curves of the same type. The amount of action, however, varies in a remarkable manner, the strong acids such as hydrochloric and nitric being less potent than equivalent concentrations of the weaker citric and oxalic acids. At N/10 concentration hydrochloric and nitric acids behave very similarly and bring out less phosphate than N/10 sulphuric acid: this in turn brings out less than N/10 citric acid, while N/10 oxalic acid gives the highest results of any.

3. When curves obtained for different periods of time are plotted together they are seen to be very much alike, but they do not all lie parallel to one another: there is a certain amount of crossing, *i.e.* the amount of action is not proportional to the time. At the beginning of the curve, where the concentration of the acid is nearly N/20, the action usually shows the remarkable peculiarity that it is *less* after 24 hours than after 10 minutes. Beyond certain concentrations,

however, the curves cross one another, and the action finally increases with the time.

4. Thus if acid of sufficiently low concentration (N/20 to N/10) is allowed to act on soil for different periods of time the amount of phosphorus compound extracted does not increase with the time but decreases, so that less is extracted after 24 hours than after 10 minutes.

This result indicates that a reverse reaction is coming into play removing the phosphorus compounds from the solution, but that it operates more slowly than the direct action of the acid in dissolving the phosphorus compounds from the soil. Thus in the 10 minutes experiment the net result is mainly determined by the direct action; after 24 hours the reverse reaction has become more pronounced and reduced the amount of phosphorus left in the solution.

5. The reverse reaction does not appear to be due to any precipitating out of the phosphorus compounds from the solution by any substance slowly extracted from the soil. For no precipitation of phosphorus compound occurs when an extract obtained by shaking soil with nitric acid for 10 minutes, and therefore rich in phosphorus, is mixed with one obtained after 24 hours' shaking, and therefore rich in any phosphorus-precipitating compound, if such is present. The seat of the reverse action, therefore, is not in the solution, but in the soil.

6. The phenomena can be reproduced by adding sodium phosphate to the mixture of soil and acid. Some of the phosphate is absorbed by the soil, notwithstanding the presence of excess of acid. The absorption is found to take place in presence of both N/10 and N/5 acid, though it fell off at the higher concentration (Table VII). Thus, the reverse reaction of § 4 is not confined to acids of low concentration, but is general: with acids of low concentration the absorption is great relative to the extraction of the phosphoric oxide; with acids of higher concentration it is small relative to the amount of P_2O_5 extracted.

7. The absorption of the P_2O_5 ion from solution in presence of acid is found to follow precisely the ordinary lines of adsorption by colloids, and is completely expressed by the ordinary adsorption formula $\frac{y}{m} = Kc^{\frac{1}{p}}$, where $y =$ the amount adsorbed by a quantity m of the soil, $c =$ the concentration of P_2O_5 in the solution when equilibrium is established, and K and p are constants (Figs. 3, 4 and 5). In one respect only is there any notable difference: adsorption by colloids is usually an instantaneous process, whilst the reverse reaction observed

in thé soil becomes more marked after 24 hours than after shorter periods. It is shown later, however (§ 15), that the discrepancy is not real. We are therefore justified in speaking of the absorption as an *adsorption*.

8. The different acids have markedly different effects on the adsorption of phosphates from solutions of sodium phosphate. Adsorption goes on readily in the presence of hydrochloric and nitric acids, but is notably smaller in presence of citric acid in equivalent concentration. It may be inferred, therefore, that the greater net action of citric acid in comparison with hydrochloric or nitric acids is not due so much to a greater solvent power, but to a greater power of reducing adsorption. Thus we should expect the actual solvent action of these acids to be more nearly alike than is indicated by the net action.
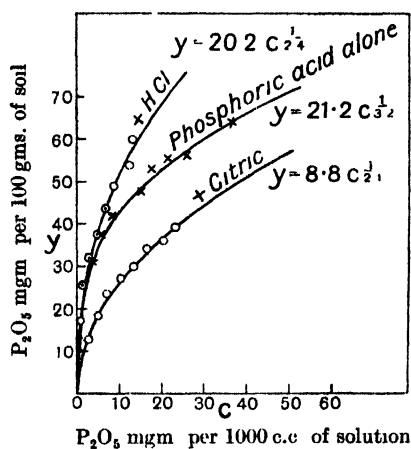
Fig 3 *a*

Fig 3 *b*.

Fig 3 *a*   Amounts of P_2O_5 adsorbed by soil Agdell B in presence of different acids.
The log curves are given in Fig 3 *b* (see p. 95).
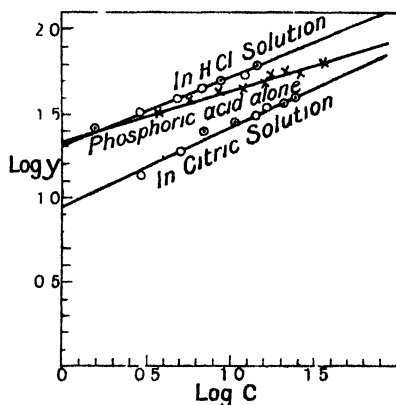
9. This expectation turns out to be correct. Adsorption can be eliminated almost entirely by arranging the experiment so that the phosphorus compound is removed from the soil as soon as it is dissolved. This is readily accomplished by diffusion. The practical difficulties are overcome by shaking the soil with 2 % agar solution, and pouring the suspension quickly into glass tubes so as to cast it into sticks. These are then placed upright in beakers containing the various acids: diffusion of acid into the stick, and of dissolved P_2O_5 out of it, rapidly takes place, and after some ten or more changes the diffusate is practically free from phosphate. Under these circumstances the amounts of phosphorus compound dissolved out from the soil are substantially

the same for hydrochloric, nitric, and citric acids (Table VIII). * Sulphuric acid, however, brings out rather higher quantities.

10. Thus the reaction of the soil phosphorus compounds with dilute acids may be resolved into two separate actions: a direct action of the acid on the phosphorus compound, and an adsorption of the dissolved P_2O_5 by the soil. In high acid concentrations the former action predominates, but both actions always go on. The solvent action is practically the same for nitric, hydrochloric, and citric acids of equivalent strengths, and appears to be the normal action of an acid on a phosphate. The reverse reaction is the typical adsorption shown by colloids, and can be expressed by the equation which has been found to fit so many of them. It is considerably influenced by the acid, being greater in the presence of the mineral acids than of the organic acids. The amount of phosphorus compound actually brought out is the difference between the direct and the reverse action. Thus hydrochloric acid dissolves out a certain amount of phosphate, but considerable adsorption takes place, so that the net amount left in solution becomes small. Citric acid dissolves out the same amount of phosphate, but there is much less adsorption, and therefore the amount left in solution is markedly greater. The difference between the various dilute acids lies, therefore, not so much in their solvent power, which is very similar for all, but in their influence on the adsorption process.

11. Since all these dilute acids behave similarly in their direct action, and differ only in the extent to which they influence the adsorption process, the observed net effect of the acid on the soil is expressed by the ordinary adsorption curve, *i.e.* parabolic curves of the general type:

$$y = Kx^{\frac{1}{p}},$$

where K and p are constants for each set of conditions, and do not include the variables x and y. But the numerical values of K and p depend on the nature and concentration of the acid, the time and temperature of the action, etc. A complete expression of the action of a given acid at varying concentrations for a given time therefore requires a series of curves, one for each concentration: or, in other words, a surface; the three variables being: P_2O_5 left in the soil, P_2O_5 left in solution, concentration of acid. The surface only expresses the action for the given time and temperature, and a series of surfaces is required to express the action at varying times but constant temperature, while with varying temperatures the case becomes more complex still. A very

pretty problem thus opens out, with which, however, we do not at present propose to deal.

The adsorption process.

12. Adsorption is not confined to phosphoric acid. Both oxalic and citric acids are adsorbed even in presence of nitric acid. Adsorption curves closely agreeing with the equation can be obtained for oxalic acid (p. 124) but not readily for citric acid, owing to the difficulty of the analytical process. On the other hand hydrochloric and nitric acids are not perceptibly adsorbed.

As a general rule adsorption of phosphoric acid is less in presence of those acids which are themselves adsorbed, than of those which are not.

We may infer that acids such as citric and oxalic satisfy the adsorption capacity of the soil and leave it with little power to take up phosphoric acid: nitric and hydrochloric acids, however, do not, and thus leave the soil free to take up phosphoric acid.

13. The order in which acids are adsorbed by soil is as follows:

$$\left.\begin{array}{l}\text{Oxalic}\\\text{Citric}\end{array}\right\}\text{ most}$$

Phosphoric
Sulphuric

$$\left.\begin{array}{l}\text{Hydrochloric}\\\text{Nitric}\end{array}\right\}\text{ least}$$

It must not be supposed that adsorption is exclusive, *i.e.* that citric acid is wholly taken in preference to phosphoric. Both are adsorbed simultaneously, but citric acid displaces some of the phosphoric.

This order appears to be determined by the nature of the acid, and not by the soil, for it is practically identical with that given by Skraup[1] for the adsorption of acids by filter paper, viz.:

Phosphoric
Sulphuric
Nitric
Hydrochloric
Acetic

When an acid is adsorbed by soil it not only displaces some of the acids below it, but also other adsorbed material   Thus, citric and oxalic

[1] Vienna, 1909, quoted in *Jour. Phys. Chem.* 1914, p. 387.

acids yield dark coloured solutions containing organic matter previously adsorbed by the soil; sulphuric acid yields lighter coloured solutions containing less; hydrochloric and nitric acids, on the other hand, displace very little organic matter, and yield almost colourless solutions. Citric acid brings out more iron than either hydrochloric or nitric acids.

14.   There is not, however, a rigid parallelism between the displacement of adsorbed organic matter and that of adsorbed phosphate. Thus, of all acids and salts investigated, ammonium oxalate gave the darkest coloured solutions, but it did not dissolve most phosphate. Again, sodium fluoride gave a dark coloured solution, but dissolved only little phosphate.

The change in adsorption with the time.

15.   The amount of adsorption depends not only on the acid but on the time.   Figs. 4 and 5 show that the continued action of N/10 nitric acid causes increased adsorption both by Hoos and Agdell soils. At first sight N/5 acid appears to show the contrary behaviour, but on closer inspection it becomes clear that the curves will cross, so that the 24 hour will ultimately come above the one hour as in the N/10 curve.   And in point of fact the N/10 curves themselves also show this relationship in the early part of their course.

Citric acid, however, behaves differently : the adsorption after one hour is greater than after 24 hours, and it is not obvious from inspection that the curves will ever cross.

It might be supposed from the N/10 nitric acid curve that adsorption was a slow business.   This does not appear to be the case, however After any given interval a definite equilibrium is attained expressed by an equation with definite constants.   The phenomena are entirely consistent with the view that the adsorption itself is instantaneous (as is almost invariably the rule with the other adsorbents), but that the constants change with the time.   In the curves of Figs. 4a and 5, p tends to fall as the time increases, so that the curve opens out.

16.   The changes in the amount of adsorption occur simultaneously with a change in the soil and in the solution.   The acid causes continuous decomposition of the soil, the extent of which may be seen from the reduction in strength of the acid.   Table III shows the amounts of nitric acid neutralised by soil after different intervals : these are considerably greater than corresponds with the calcium carbonate, phosphates, etc., present.   Much silica is liberated during the action.

Agdell soil.

$y=9\,4\,c^{\frac{1}{1\cdot5}}$

24 Hrs

1 Hr
$y=11\,3\,c^{\frac{1}{4}}$

Time = 0
$y=11\,5\,c^{\frac{1}{4\cdot1}}$

P₂O₅ mgm. per 1000 c.c. of solution
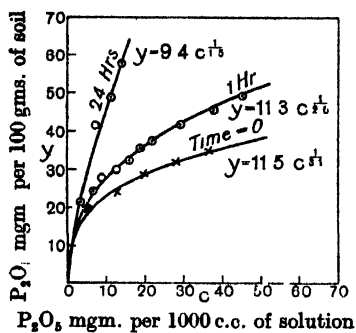
Fig. 4 a.

Agdell soil, log. curves.

24 Hrs

1 Hr

Log y

Log C

Fig. 4 b.

Saxmundham soils.

Unmanured
$y=6\,3\,c^{\frac{1}{2\cdot6}}$

Superphosphates
$y=7\,9\,c^{\frac{1}{3}}$

P₂O₅ mgm. per 1000 c.c of solution
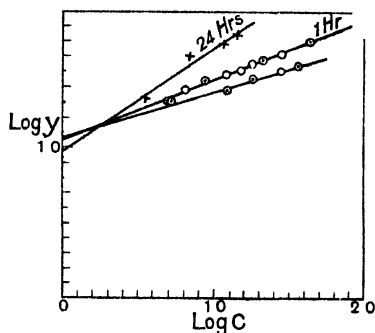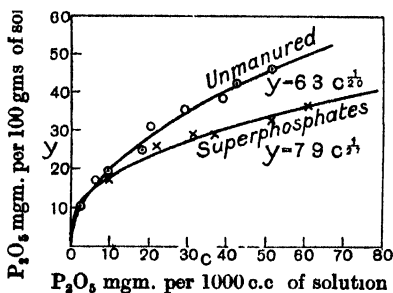
Fig. 4 c

Saxmundham soils, log. curves.

Unmanured

Superphosphate

Log y

Log C

Fig 4 d.

Light soils.

Woburn
$y=30\,7\,c^{\frac{1}{8\cdot2}}$

Bramford
$y=2\cdot7\,c^{\frac{1}{1\cdot6}}$

P₂O₅ mgm per 1000 c.c. of solution
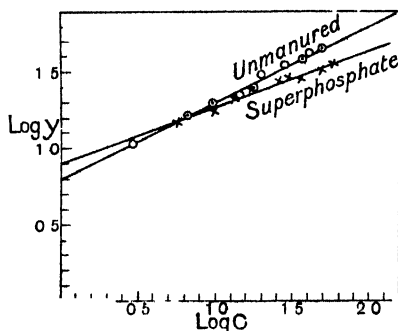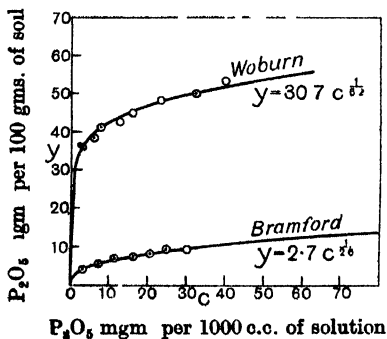
Fig. 4 e.

Light soils, log. curves.

Woburn

Bramford

Log y
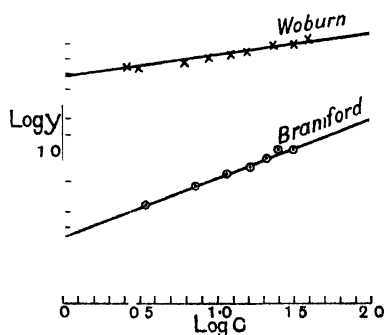
Log C

Fig 4 f.

Fig. 4 a, c and e. Amounts of P₂O₅ adsorbed by various soils in presence of N/10 HNO₃.
Fig. 4 b, d and f. The log. curves.
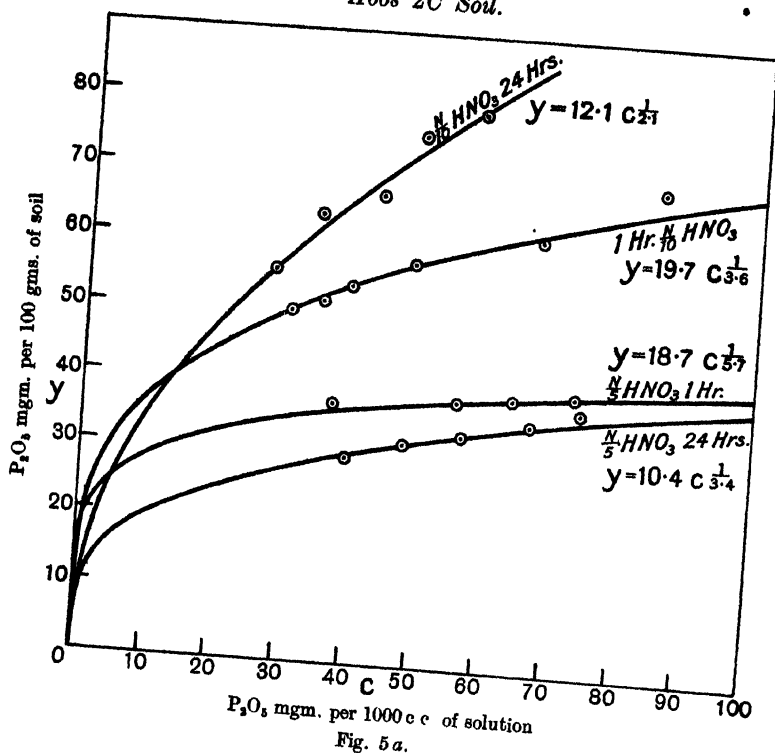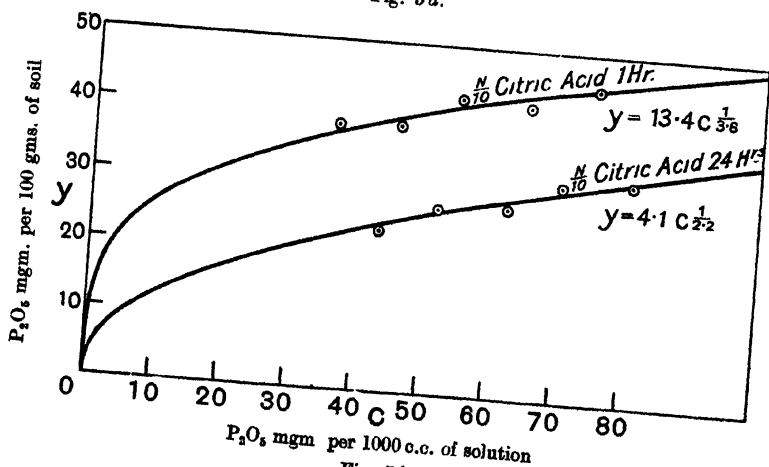
Fig. 5. The effect of time, and of strength of acid, on the adsorption phenomena.

17. It does not appear, however, that treatment with acid affects the adsorptive capacity of the soil: the curve obtained after preliminary treatment of the soil with acid coincides with that for the untreated soil (Fig. 6).

Agdell Soil.
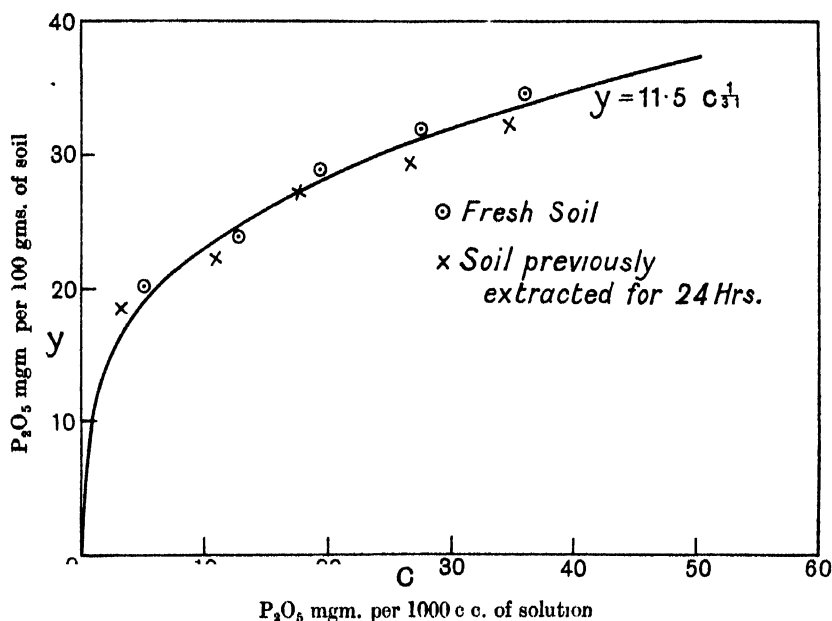
Fig. 6. Adsorption of P_2O_5 in presence of N/10 HNO_3 from (*a*) fresh soil, (*b*) soil previously extracted for 24 hours by N/10 HNO_3.

The change in the constants with the time appears to be determined by the changes in the solution. Some soil was divided into two parts: one was treated with acid which had been in contact with soil for 24 hours, and the other with an equivalent amount of fresh acid. The adsorption curves were entirely different: that obtained with the fresh acid falling below the other, and being related to it in the same manner as the "Time = 0" curves are to the "Time − 24 hour" curves (Fig. 7).

It follows that the simplest results are obtained when the time is reduced to a minimum, because here the composition of the liquid has suffered minimum change by interaction with the soil.
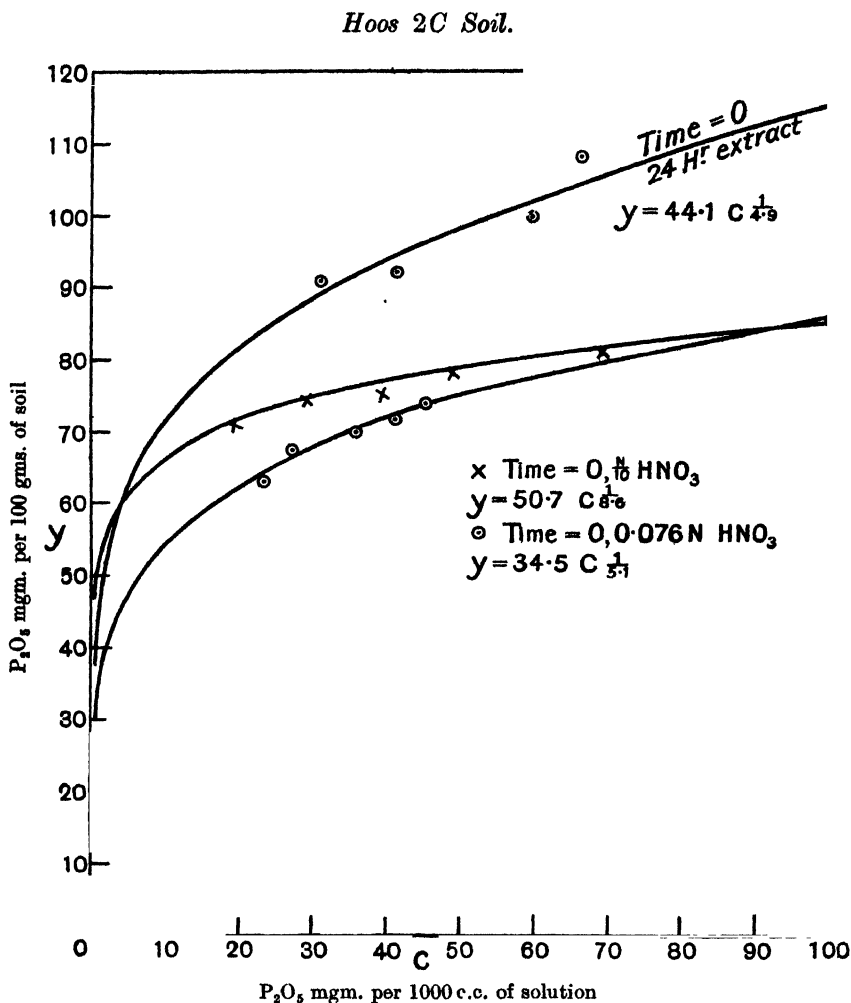
Fig. 7. Adsorption of P_2O_5 in presence of (a) N/10 HNO_3 which has already been in contact with soil for 24 hours, (b) fresh HNO_3 of equivalent concentration. Two experiments were made, one with N/10 acid (equivalent initial concentration), the other with 0·076 N HNO_3 (equivalent final concentration).

The equations obtained and the meaning of the constants.

18. The following equations have been obtained expressing the equilibrium obtained in the adsorption of P_2O_5 by soils in the presence of various acids:

Heavy soils. Rothamsted

General equation $y = KC^{\frac{1}{p}}$

N/10 HNO$_3$	Time = 0	Time = 1 hr.	Time = 24 hrs.
Hoos 2 C (Superphosphate)		$y = 19 \cdot 7\, C^{\frac{1}{3 \cdot 6}}$	$y = 12 \cdot 0\, C^{\frac{1}{2 \cdot 1}}$
Agdell (unmanured)	$y = 11 \cdot 5\, C^{\frac{1}{3 \cdot 1}}$	$y = 11 \cdot 3\, C^{\frac{1}{2 \cdot 6}}$	$y = 9 \cdot 4\, C^{1 \cdot 5}$
„ „ *		$y = 16 \cdot 1\, C^{\frac{1}{5 \cdot 0}}$	
„ „ extracted†	$y = 11 \cdot 5\, C^{\frac{1}{3 \cdot 1}}$		
N/5 HNO$_3$			
Hoos 2 C		$y = 18 \cdot 7\, C^{\frac{1}{5 \cdot 7}}$	$y = 10 \cdot 4\, C^{\frac{1}{3 \cdot 4}}$
N/10 HCl			
Agdell B (extracted)‡			$y = 20 \cdot 2\, C^{\frac{1}{2 \cdot 4}}$
N/10 citric acid			
Hoos 2 C		$y = 13 \cdot 4\, C^{\frac{1}{3 \cdot 6}}$	$y = 4 \cdot 1\, C^{\frac{1}{2 \cdot 2}}$
Agdell B (extracted)†			$y \quad 8 \cdot 8\, C^{\frac{1}{2 \cdot 1}}$

Lighter soils. Saxmundham.

N/10 HNO$_3$. Time = 2 hours

Plot 4 (2 cwt. superphosphate annually) ...   . $y = 7 \cdot 9\, C^{\frac{1}{2 \cdot 7}}$

Plot 6 (unmanured) ...   . $y = 6 \cdot 3\, C^{\frac{1}{2 \cdot 0}}$

Woburn.

N/10 HNO$_3$. Time = 1 hour

Stackyard wheat plots (unmanured)   ...   . $y = 30 \cdot 7\, C^{\frac{1}{6 \cdot 2}}$

Lightest soil. Bramford§.

N/10 HNO$_3$. Time = 1 hour

...   . $y = 2 \cdot 7\, C^{\frac{1}{2 \cdot 6}}$

* Na$_2$HPO$_4$ added after 50 minutes' shaking. See p. 106
† With N/10 HNO$_3$ which removed 3 60 mgms. P$_2$O$_5$ per 100 gms. soil. See p 108.
‡ With H$_2$SO$_4$ and then NaOH. See p. 94.
§ Owing to the richness of this soil in phosphates it had to be subjected to a preliminary extraction with N/5 nitric acid.

With the exception of the Woburn soil these all fall into a series, K varying from 19·7 in the heavy Hoos field soil, down to 2·7 in the light Bramford soil, while y shows much less change and at equal times has actually the same value for the heavy as for the light soils.

The question naturally arises: what do these constants mean? and are they connected with any of the other properties of the soil?

At the outset it must be observed that these "constants" have no absolute value: they are not constant for the soil but only for the special conditions under which the experiment was carried out. Thus, for Hoos 2C: K varies from 4·1 to 19·7, and no doubt wider variations could be obtained. The figures, therefore, cannot be regarded as absolute measures of any soil property.

Nor can they be used indiscriminately even for purposes of comparison when different soils are being studied under the same experimental conditions. For however nearly alike the conditions may be at the beginning of the experiment they soon begin to diverge as the experiment proceeds. It is shown on p. 109 that the substances dissolved out from the soil considerably affect the values of the constant.

But by exercising due caution it is possible to learn something from them.

The meaning of K may be arrived at by inspection of the equation. It is the amount of P_2O_5 that will remain in the soil when C 1, *i.e.* when unit quantity of P_2O_5 is present in the solution; it therefore represents *the tenacity with which the soil keeps its* P_2O_5, or in other words, the reluctance with which the soil parts with its P_2O_5, under the conditions of the experiment.

It is high for heavy soils, low for light ones (except Woburn): it falls off under the continued action of the acid, so that it is less after 24 hours than after one hour; it is greater in presence of nitric and hydrochloric acids than of citric acid.

The meaning of p is less obvious. The equation shows that it is more complex than K, being the ratio of the logarithms of c and y. Inspection of the curves, and especially of Fig. 4 a, shows that where p is large the curve bends over to the axis of X, *i.e.* the soil soon ceases to adsorb P_2O_5, so that large amounts remain in the solution. Where p is small the curve opens out, and more P_2O_5 is taken up by the soil, while less remains in the solution. p, therefore, is connected with *the manner in which the adsorptive capacity of the soil is satisfied*, or in which *the soil takes P_2O_5 out of the solution* under the conditions of the experiment.

It is not much affected by the soil, but it falls off with the time of action.

It has been pointed out that the heavy soils show a high value for K, *i.e.* a high reluctance to part with their P_2O_5, while the light Bramford soil only shows a small reluctance and parts with it fairly easily. We are unable to explain why the Woburn soil behaves otherwise. The immediate reason is that the diffusion process does not come to an end as speedily as in other cases, - a phenomenon which would be readily explained if small nodules of coprolite were present,—as indeed they are known to be elsewhere in the district.

Whatever the explanation of the behaviour of the Woburn soil there is evidence that this distinction holds generally: it is difficult to get clean cut illustrations, but as a rule sandy soils respond less to phosphates than clay soils, not only because there is often more there, but also because plants can make more use of what is actually present, in other words, the sandy soils part with their phosphates more readily than heavier soils would do. This is clearly a promising field for enquiry, and we hope that fresh data will be obtained so that a fuller discussion may become possible.

It is less easy to say much about p because it varies so little with changes in the soil, and so much with changes in the solution. It is apparently less directly connected with the properties of the soil than K, and is therefore of less interest to us. And as physicists themselves have not come to any agreement as to its meaning in spite of a vast amount of work, we may safely leave it alone for the present.

The bearing of the results on soil analysis.

Since the action between dilute acids and soil consists of two parts: a direct and a reverse reaction, it follows that the amounts of P_2O_5 brought out by the ordinary treatment with dilute acids do not represent any definite extractable material in the soil, but only the difference between the quantity dissolved by the acid and that adsorbed by the soil, which varies with the acid and the conditions of the experiment.

Thus it is incorrect to call the net amount brought out the " available P_2O_5 " as if it were something real in the soil: it is simply an analytical result, which will vary when the conditions of treatment are varied. If two soils have widely different adsorptive capacities they may give different analytical results even when they contain similar amounts of phosphate. Of all the acids examined citric suffers least complication from adsorption, a fact which shows the wisdom of Dyer's choice in

1894. But all acids are affected. The only way to get precise determinations of the easily soluble phosphate in the soils is to adopt a diffusion method whereby the reverse reaction becomes eliminated.

This is perfectly feasible. But so long as the conditions of the experiment are constant and the soils are of the same character, possessing similar adsorptive capacities, dilute acids may be expected to give comparable results. Not otherwise, however.

Comparable results may be of great value to soil analysts. Obviously they can be obtained only by first selecting typical soils and determining the data for them as fully as can be. This involves a soil survey, and is one of the many justifications that can be urged in favour of surveys. When typical soils have been studied it is not difficult to compare any soil *of the same kind* with them, and to give information of value to the farmer; but our results show the hopelessness of trying to compare two dissimilar soils.

Absolute results could be obtained by the diffusion method, but until we have had more experience with it we are not prepared to say what sort of value they would have for the analyst.

EXPERIMENTAL.

The soils and methods used.

Heavy soils. Rothamsted. Heavy soil, Clay-with Flints formation.

Agdell field. This is under a rotation,—clover, wheat, swedes, and barley, and has been for many years. The soil was taken from the space between the plots unmanured since 1843, and has neither carried crops nor received manure.

Hoos field. Continuous barley plots. 1.0.- Unmanured since 1852. 2C.—This has received 1000 lbs. of rape cake, and 3½ cwts superphosphate each year since 1852.

Lighter soils. Saxmundham. A Boulder Clay from the Experimental field of the East Suffolk County Council.

Rotation I. Plot 6 — Unmanured since 1900. Plot 4.—This has received 2 cwts. superphosphate each year since 1900.

Woburn. Lower greensand formation. From the continuous wheat plots in Stackyard field of the Royal Agricultural Society's farm. This plot has been unmanured since 1872.

Lightest soil. Bramford. A light gravel soil from the farm on which the East Suffolk County Council experiments were carried out during the years 1893 to 1910.

The mechanical and chemical analyses of these soils are given in Table I.

TABLE I. *Mechanical analyses of soils.*

	Heavy soils, Rothamsted		Lighter soils			Lightest soils
	Agdell	Hoos Field	Saxmundham		Woburn	Bramford
		Plot 2C	Plot 6	Plot 4		
Fine gravel	0·4	0·7	2·1	0·7	0·4	1·0
Coarse sand	6·4	11·3	41·5	38·6	58·0	50·3
Fine sand	29·1	22·5	15·9	18·1	13·4	23·6
Silt	21·6	24·2	4·5	5·5	7·0	2·2
Fine silt	11·8	12 8	7·1	6 6	6 9	5·5
Clay	15·4	14·0	15·7	16·0	5·7	3·9

Chemical analysis of the soils.

	Agdell	Hoos 2C	Saxmundham		Woburn	Bramford
			6	4		
Phosphoric acid (P_2O_5) soluble in strong HCl	0·110	0·255	0·096	0·096	0 121	0·17
Phosphoric acid (P_2O_5) soluble in 1 % citric acid	0·012	0·083	0·009	0·014	0·011	0·11
Potash (K_2O) soluble in strong HCl	0·520	0·503	0·568	0·575	0·24	—
Potash (K_2O) soluble in 1 % citric acid...	0·012	0·014	0·008	0·009	0·021	—
Calcium carbonate	0·04	0·14	0·31	0·37	nil	—
Loss on ignition	4 9	5·0	5·1	—	3 5	4·1

The method of extraction of the soil with the acid. A special feature of our experiments is that the extraction was carried out at constant temperature. The apparatus is shown in Fig. 8. A bicycle wheel is mounted at the apex of a triangular wooden stand, which can either rest on the floor, holding the wheel upwards, or else on the top rim of the thermostat, holding the wheel downwards in the water. The wheel is fitted with stout wooden clips enabling it to carry eight Winchester pint bottles. As a preliminary to each experiment 100 gms. of soil and a volume of distilled water sufficient to make one litre of dilute acid are placed in each bottle, which is then closed with a rubber stopper; the bottles are fixed on the wheel, put into the thermostat, and rotated by means of a hot air motor for an hour, by which time the contents have attained the temperature of the thermostat (23° C.). Then the bottles are lifted out, the requisite quantity of strong acid (2 N) is added
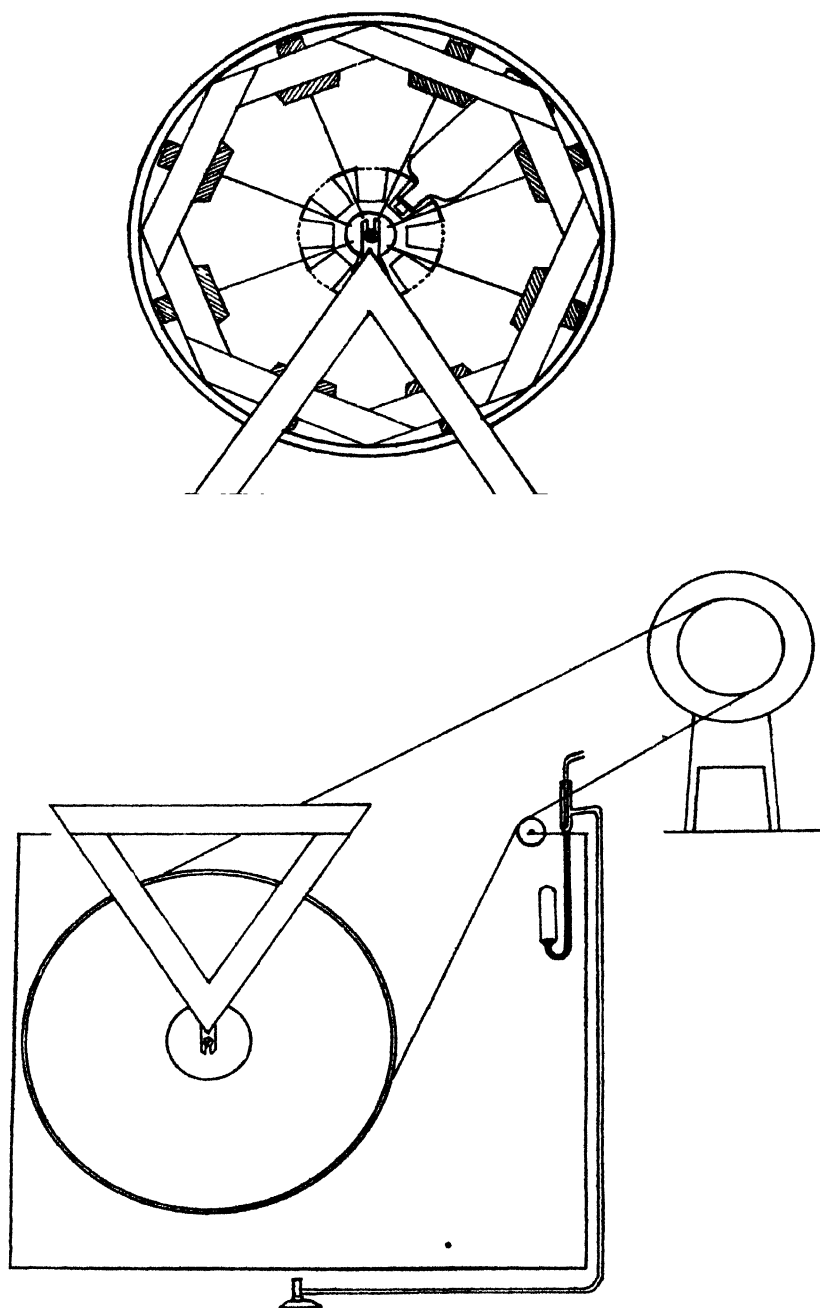
Fig 8   Apparatus used for shaking soil with acid at constant temperature

to each, and they are rapidly replaced in the thermostat and shaken for the proper time. In the case of the so-called "Time = 0" experiments the bottles were not put back, but were vigorously shaken for a few seconds and allowed to stand a further few seconds for settling.

The estimation of the phosphate.

The liquid is rapidly filtered through asbestos packed on a Buchner funnel; 250 c.c., or if necessary 500 c.c., of the filtrate are evaporated to dryness, and the residue treated as already described by one of us[1].

The action of dilute acids on soils. Relation between the concentration of the acid and the amount of P_2O_5 extracted.

Schloesing and de Sigmund have both stated that the amount of P_2O_5 extracted from a soil by acids of increasing concentration is constant over part of the curve, but we failed to find evidence of this. Working exactly as they did, viz. at laboratory temperature, we obtained the results given in Table II, and plotted in Fig. 1, which certainly might be made to fit a curve like theirs, but could equally fit any other. Under the more precise conditions of the thermostat we obtained the smoother results given in Table III, and plotted in Fig. 2.

In all cases there was a considerable excess of acid left after the experiment, though the amount that had been neutralised was considerably more than corresponds with the calcium carbonate (which in this case was only small) and the P_2O_5 extracted.

The action is complex: aluminium and silica are both found in solution and in increasing quantities as the action is prolonged. But the final solution is always strongly acid, and readily dissolves calcium phosphate and attacks fresh soil dissolving out more phosphate: the variations in amount of P_2O_5 dissolved cannot therefore be attributed to exhaustion of the acid.

The amounts of P_2O_5 extracted by sulphuric, citric, and hydrochloric acids are given in Table III, and plotted in Fig. 2. They increase with the concentration of the acid, but not quite proportionately, and the curves are apparently not straight lines over their whole course, although they usually are nearly so. There is no sign of a break anywhere, and in particular no indication of the horizontal run shown in de Sigmund's

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[1] Prescott, This *Journal*, 1914, **6**, 110. Extended experience with this method has demonstrated its great accuracy for small amounts of P_2O_5 such as occur in soil extracts

curve, on which was based the evidence for simple dissolution of a simple phosphate.

TABLE II. *Amounts of* P_2O_5 *extracted by dilute nitric acid of varying concentration from soil (Barnfield Subsoil) in 24 hours at room temperature ;* 100 *gms. of soil to* 1000 *c.c. of acid.*

Initial acidity Equivalents HNO_3 per litre	Mgms. P_2O_5 dissolved per 100 gms. of soil			
	(1)	(2)	(3)	(4)
·08	0 5	1 1	0·7	0·4
·12	2·2	2·0	1·9	—
·13	2·3	2·1	2·4	1·9
·14	2·8	—	2 3	—
·15	2 7	2 3	—	2·7
·16	3·1	2·5	2·8	2·8
·17	3·4	3·4	2·9	3·7
·18	3·6	4·2	3·5	3 7

In 1 *hour at room temperature.*

Initial acidity Equivalents HNO_3 per litre	Mgms P_2O_5 dissolved per 100 gms. of soil	
	(1)	(2)
06	2·0	1·9
·08	3·1	3·2
·10	4·0	3·7
·12	4·4	3·8
14	5 7	4 9
·16	6 1	4·9
·18	5 8	6·3
·20	7·2	

The effect of time on the process. This is shown both in the Tables and the Curves. Nitric acid gives the most remarkable result of all. The shorter the time of extraction the more P_2O_5 is dissolved: in a few seconds more is brought out than in 20 minutes, and considerably more than after 24 hours. This holds for all strengths of acids up to N/5, and beyond with poor soil, though with the richer Hoos 2C soil the curves cross at N/8 (Fig. 2 e). The result can only mean that P_2O_5 is being taken back from the solution by the soil.

Hydrochloric acid shows the same behaviour up to N/10, but at greater concentrations the curves cross; at N/5 the 24 hour curve lies

TABLE III. *Amounts of* P_2O_5 *extracted from soil by* 1000 *c.c. of acids of different strength at constant temperature.*

Agdell Soil.   *Temperature.* 23° *C.*

	Sulphuric acid			Citric acid		
Initial acidity Equivalents H_2SO_4 per 1000 c.c.	Mgms. P_2O_5 per 100 gms. of soil		Initial acidity Equivalents citric acid per litre	Mgms P_2O_5 per 100 gms. of soil		
	(a)	(b)		(a)	(b)	
Time = 0			Time = 1 hour			
·057	7 1	6·6	·06	6·2	5·9	
·114	7·8	8 4	·12	6·9	6·7	
·170	8·5	9·7	·16	7·0	7·4	
Time = 2 hours			·20	7·6	8·0	
·06	8 0	8·1	Time = 24 hours			
·10	—	12·1	·06	9·7	10 1	
·14	15·1	14·9	·12	11·0	11·3	
·18	17·1	17·9	·16	11 3	11·7	
Time - 24 hours			·20	12·1	12·3	
·057	4 2	4 2				
·114	11·8	11 4				
·170	17 2	17·4				

Note. Extractions were also made with acetic acid, but the amounts of P_2O_5 were too small to be estimated.

Hydrochloric acid

Initial acidity Equivalents HCl per litre	Mgms. P_2O_5	
	(a)	(b)
Time = 0		
·06	2 5	2·6
·12	3 8	3·5
·18	4 6	4·6
Time = 2 hours		
·06	2·5	—
·10	3·2	3 0
·14	4·1	4·7
·18	5·4	5·4
Time - 24 hours		
·075	1 3	1·7
·149	3·7	4·0
·224	6·5	6·4

TABLE III (*continued*). *Amounts of P$_2$O$_5$ extracted from Soils by* dilute Nitric Acid *of varying strength acting for different periods of time.*

Aydell Soil, 100 *gms to* 1000 *c.c of acid.*   *Temperature* 23° C.

Initial acidity Equivalents HNO$_3$ per litre	Mgms P$_2$O$_5$ extracted								
	0		0 33 hrs		2 hrs	9 hrs		24 hrs	
	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*a*)	(*b*)	(*a*)	(*b*)
06	3 7	—	3 0	3 6	2 3	1 5	1·6	0 7	1 0
08	4 3	—	3 5	4 1	2 9	2 0	2 1	2 1	1 4
10	5 2	—	3 6	5 1	3 5	2 5	—	1 2	2 6
12	5 5	6 2	4 9	5 0	3 8	3 0	3 0	2 5	2 3
14	6 0	6 1	5 6	5 6	4 0	3 6	3 5	3 3	3 2
16	6 0	6 2	6 0	—	4 8	3 6	4 0	3 4	2 7
18	6 5	6 1	6 4	—	4 6	—	4 9	4·1	4 5
20	6 9	6 3	6 8	—	5 7	—	4 9	4 6	3 9

Equivalents of acid neutralised *in the above experiments*

Initial acidity Equivalents HNO$_3$ per litre	Amount of acid neutralised by soil after				
	0	0 33 hrs	2 hrs	9 hrs	24 hrs
06	0160	0182	0206	0236	0270
08	0156	0196	0224	0252	0286
10	0166	0206	0242	0280	0304
12	0174	0216	0244	0304	0330
14	0182	0218	0262	0324	0338
16	0182	0232	0276	0326	0352
18	0194	0240	0284	0246	0368
20	0196	0252	0288	0356	0376

Hoos 2 C' (50 *gms. used per* 1000 *c c of acid but results calculated to* 100 *gms*)

Initial acidity Equivalents HNO$_3$ per litre	Mgms P$_2$O$_5$ per 100 grams of soil	Initial acidity Equivalents HNO$_3$ per litre	Mgms P$_2$O$_5$ per 100 grams of soil
Time 0 33 hours		Time =4 hours	
06	47 6	14	59 2
10	46 7	18	64 1
·12	55 1	Time — 24 hours	
·14	55 6	06	33 0
·18	59 2	10	44 9
Time — 4 hours		12	55 8
06	35 8	·14	60 1
10	56 2	18	72 8
·12	54 0		

above the Time -- 0 curve, and is approaching, and shows signs of soon crossing, the 2 hour curve. Sulphuric acid behaves in the same way, but the points of crossing occur at still lower concentrations; the 2 hour curve has already crossed the Time -- 0 curve at concentrations lower than those we used, while the 24 hour curve crosses it just before N/10. At higher concentrations the 24 hour curve crosses the 2 hour curve, and at N/5 the longer the time of action the greater the amount of P_2O_5 extracted. Citric acid always gives this result at all of the times and concentrations used by us, but we have not tried extremely dilute acids.

In comparing the various acids, therefore, a steady gradation can be detected: nitric acid shows the remarkable falling off in net action, and therefore an increase in the reverse action with the time at all concentrations up to N/5 and apparently beyond, hydrochloric acid shows it nearly up to N/5; sulphuric acid only up to N/10; while citric acid either does not show it, or if it does, only at concentrations below N/20.

This marked difference in behaviour between citric and nitric acids is not due to any special decomposition effected by citric and not by nitric acid, for after the soil has been treated 21 hours with citric acid it behaves in the same way towards nitric acid.

A quanitty (300 gms.) of Agdell soil was extracted for 24 hours with N/10 citric acid. It was then divided into three lots: one was extracted with HNO_3 for half an hour; the second for 21 hours; the third with N/10 citric acid for 24 hours. The results were as follows:

1st extraction, N/10 citric acid
10 4 mgms P_2O_5 per 100 gms of soil

2nd extraction

N/10 HNO_3 Half-an-hour	N/10 HNO_3 24 hours	N/10 citric acid 24 hours
3·5	1·7	3·8 mgms. P_2O_5 per 100 gms. of soil

The half-hour treatment with nitric acid still brings out more P_2O_5 than the 24 hours' treatment.

The falling off in solvent action with the time is not peculiar to our soil. Surprisingly few out of the vast number of soil analyses bear on

the point: we have, however, found a paper by Lemmermann[1] in which similar results were obtained: dilute nitric acid dissolving out more P_2O_5 from the soil in $1\frac{1}{2}$ hours than in 72 hours.

His results are:

Soil from	Mgms P_2O_5 dissolved per 100 gms. of soil in		Ratio $1\frac{1}{2}$ hrs. 72 hrs.	Clay in soil* per cent.
	$1\frac{1}{2}$ hours	72 hours		
Dahlem . .	7·3	7·3	1·00	3·7
Boche.........	1·4	1·2	1 17	4·3
Prüfer	12 3	8 4	1·46	11·1
Marsch	8·4	5 0	1 68	18 1
Rettgau....	3·6	0·9	4·00	31·3

* Ascertained by J. A. Prescott in 1913.

The effect varies in the different soils. The Rettgau soil is very heavy, and the Dahlem soil very light. One of us ascertained the percentage of clay in the soils, and, it will be observed that the falling off of the solvent action with the time,- in other words the extent of the reverse reaction, —varies with the percentage of clay in the soil.

The effect of different dilute acids. The results given in the different parts of Table III are all comparable, and lead to the following conclusions:

Nitric acid shows the least net solvent effect, and the greatest reverse effect.

Hydrochloric shows more net solvent effect, and less of the reverse effect.

Sulphuric shows still more of the net solvent effect, and still less of the reverse effect.

Citric acid shows the most net solvent effect, and the least reverse effect, the acids being all in equivalent concentrations.

Beyond certain concentrations and times, however, sulphuric acid has a greater net solvent effect than citric acid.

The extent of the net reaction therefore varies inversely with that of the reverse reaction.

The nature of the reverse reaction. The reverse action by which P_2O_5 is removed from the solution is not a precipitation caused by a change in composition of the liquid. This was proved by adding the

[1] *Landw. Versuchs-Stat.* 1913, **83**, 357.

24 hour extract to the instantaneous nitric acid extract, which contained a high amount of P_2O_5; no precipitation occurred. That the soil and not the extract was responsible for the removal of the P_2O_5 was demonstrated by adding sodium phosphate to the mixture of soil and nitric acid; in spite of the excess of acid some of the added P_2O_5 was withdrawn from the solution by the soil (Table IV).

TABLE IV. *Amounts of P_2O_5 removed by soil from sodium and calcium phosphates in free $N/10$ nitric acid*

Mgms. of P_2O_5 added in Na_2HPO_4	Mgms of P_2O_5 found in the nitric acid extract	Mgms. of added P_2O_5 recovered	Percentage of P_2O_5 recovered
0	2·9	—	—
6·6	6·7	3·8	57·0
22·0	18·3	15·4	69·6
49·6	39·2	36·2	73·1
66 2	51 8	48·9	73·8

	P_2O_5 added, mgms.	P_2O_5 recovered, mgms.	Percentage of P_2O_5 recovered
Soil + 0·1 gms. apatite	41·3	20·6	49·9
Soil + 0·1 gms. tricalcic phosphate	41·1	22·2	54·0
Soil + 0·1 gms dicalcic phosphate	53·7	28·2	52·5

In all cases excess of acid remained at the end of the experiment.

In view of the excess of acid invariably present the removal of the P_2O_5 from the solution cannot be regarded as simple chemical precipitation.

There remained the possibility that the action might belong to the remarkable physical effects classed vaguely as absorptions in the older days, and now called by the more definite name of adsorptions.

It is well known that charcoal has the power of withdrawing certain dissolved substances from their solutions. There is no evidence of any chemical change in the ordinary sense of the term, and indeed the quantitative relationships are quite different from those of any ordinary reaction. A considerable amount of experimental work has shown that the quantity of substance adsorbed by a given amount of the adsorbent is not proportional to the actual amount present in the solution, but to some power of this quantity: so that if

$y =$ amount of substance adsorbed,

$c =$ concentration of the substance in the solution when equilibrium is reached,

$m =$ amount of adsorbent,

then $\dfrac{y}{m} = Kc^{\frac{1}{p}}$, where K and p are both constants.

This curve is parabolic: it becomes the ordinary parabola in the special case where $p = 2$.

This curve has been found to hold for the most diverse cases; for blood charcoal and various acids, chlorine, bromine, etc.; for wool and dyes; for filter paper and acids; and numerous others. It does not hold invariably, however, which indicates that there may be various types of adsorption, but it stands for the most usual type.

In applying this equation to the case in hand we are confronted with the difficulty of determining y,– the amount of P_2O_5 adsorbed. It is, of course, easy to find out what proportion of added P_2O_5 is adsorbed (as shown in Table IV) but this takes no account of the adsorption of the P_2O_5 given up by the soil to the solution.

Two methods were therefore adopted.

1. The extractable P_2O_5 was carefully removed from a sample of Agdell field soil by extracting it twice with N/5 H_2SO_4, and then seven times with 2 % caustic soda in the cold. The resulting material gives up no P_2O_5 when treated with dilute acids. It was shaken with a mixture of HCl (0·06 N) and sodium phosphate for 24 hours at 23 C.

Direct analysis of the resulting solution gives c in the above equation: subtraction from the amount originally added gives y. there is here no complication from the amount initially present in the soil because all this was removed in the preliminary treatment. The results are given in Table V. When these values are substituted in the equation it is found that

$$K = 20\text{·}2; \quad p = 2\text{·}4 \text{ for the hydrochloric acid experiment}$$

and $\quad K = 8\text{·}8; \quad p = 2\text{·}1 \text{ for the citric acid experiment.}$

Thus the two equations become

$$y = 20\text{·}2\, C^{\frac{1}{2\text{·}4}}$$

and $$\quad\quad y = 8\text{·}8\, C^{\frac{1}{2\text{·}1}} \text{ respectively.}$$

The curves are drawn on Fig. 3: it is clear that they fit the experimental points very well.

A simpler and more accurate method of checking the results and observing the agreement is to take out the logarithms of y and c

If the equation is true.

$$\log y = \frac{1}{2 \cdot 4} \log C + 1 \cdot 3$$

i.e.

$$\log y = \frac{1}{2 \cdot 4} \log C + 1 \cdot 3$$

TABLE V   *Adsorption of P_2O_5 by soil, Agdell B, from which all phosphate has been removed by extraction with H_2SO_4 followed by NaOH (p 94)*

Time 24 hours   *Temperature 23° C*

50 gms of soil to 1000 c c of solution

Phosphoric acid alone

P₂O₅ added(mgs per 1000c c)	19 6	24 7	29 6	34 5	39 5	44 4	49 3	54 3	69 1
P₂O₅ found in solution (c) (mgs per 1000 c c)		3 7	5 6	8 4	12 0	15 5	17 8	21 4	25 9	36 6
P₂O₅ left in soil (y) (mgs per 100 gms of soil)		31 8	38 2	42 4	45 1	48 0	53 2	55 9	56 7	65 0

Equation obtained $y = 21 2 C^{\frac{1}{3 \cdot 2}}$

In presence of HCl ·06 N

P₂O₅ added(mgs per 1000 c c)	9 7	14 5	19 3	24 1	29 0	33 8	39 6	43 4
P₂O₅ found in solution (c) (mgs per 1000 c c)	0 8	1 6	2 9	4 8	6 7	8 9	12 4	13 1
P₂O₅ left in soil (y) (mgs per 100 gms of soil)	17 8	25 8	32 8	38 7	44 5	49 9	54 4	60 7

Equation obtained $y = 20 2 C^{\frac{1}{2 \cdot 4}}$

In presence of Citric acid N/10

P₂O₅ added(mgs per 1000 c c)	9 7	14 5	19 3	24 1	29 0	33 8	38 6	43 7
P₂O₅ found in solution (c) (mgs per 1000 c c)	2 9	5 0	7 1	10 3	13 6	16 4	20 2	23 6
P₂O₅ left in soil (y) (mgs per 100 gms of soil) .	13 4	19 0	24 4	27 7	30 8	34 9	36 8	40 1

Equation obtained $y = 8 8 C^{\frac{1}{2 \cdot 1}}$.

The amount of P_2O_5 left in the soil is calculated to mgms per 100 gms of soil so as to facilitate comparison with the other results   The amount of soil actually used was 50 gms.; the experimental values for y were therefore one half of the figures here given.

This is the equation of a straight line. If, therefore, $\log y$ is plotted against $\log C$ all the points ought to lie on a line. The figures show that they do so.

2. Sodium phosphate was added but the soil was not subjected to any preliminary treatment; instead the amount of extractable P_2O_5 was determined. This value *plus* the amount of added P_2O_5 gives the total $y + C$. C being determined by analysis, y is known by simple subtraction.

TABLE VI. *P_2O_5 contained in successive* 24 *hour periods of diffusion in* $N/10$ *HCl. The results are calculated as a percentage of the soil.*

Soils	1	2	3	4	5	6–9	10–13
Hoos 1 O	→	→	→	→	·001	·003	·004
Hoos 2 C	·027	·0132	·0075	→	·0097	·0195	·0128
Agdell	·003	·0018	→	→	·0021	·0054	·0048
Teynham	·054	·0192	0081	→	·0112	0144	·0087
Saxmundham*	·0027	·0015	→	→	·0015	·0043	·003
„ †	·0045	·0021	→	→	·0018	·0060	0043
Woburn	·0064	·0045	·0042	→	0078	·0108	·0067

Soils	14–17	18–21	Total	HCl soluble ("Total")	Citric soluble ("available")
Hoos 1 O	trace	trace	·008	·101	·0053
Hoos 2 C	·0126	·009	·111	·255	·083
Agdell	·0048	·0030	·025	·110	·012
Teynham	·0067	·0042	·126	·172	·084
Saxmundham*	·002	·002	·017	·096	·009
„ †	·0043	·003	·026	·096	·014
Woburn	·0055	trace	·046	·159	023

* Plot 6 (unmanured).　　　† Plot 4 (superphosphate).

In determining the total extractable P_2O_5 it is obviously necessary to eliminate the reverse reaction. This is done by a diffusion process. The ordinary diffusion thimble is not satisfactory for soil, and no muslin is fine enough to hold it together and keep it out of the diffusion liquid. The soil was therefore shaken with 2 % agar solution, and then cast into solid sticks by pouring into a glass tube of 10 mm. diameter and 100 mm. in length; these sticks can be placed in dilute acids, and they allow of rapid diffusion without disintegration. In carrying out the experiment 5 to 10 gms. of soil and 30 c.c. of agar

are convenient quantities; this makes three sticks, which for better protection are put into a muslin bag; this is then suspended from a glass rod into a 250 c.c. bottle containing the acid.   Twice in each 24 hours the liquid is changed, and the whole is evaporated to dryness

The results given in Table VI show how the P_2O_5 comes out from the soil; the experiment was continued for 21 periods each of 24 hours. For comparison the "Total P_2O_5" (i e the amount soluble in boiling concentrated HCl) and the "available P_2O_5" (i e. the amount soluble in 1 % citric acid) are also given.

In practice it is unnecessary to pour off more than 10 times for citric acid, or 20 times for hydrochloric or nitric acid.   There is, of course, no need to make separate determinations: it is easier and more accurate to continue the evaporations in the same basins till the process has gone sufficiently far.

When the total extractable P_2O_5 has been determined the value is used as above for determining y, and the equation is then easily completed.

The results obtained with five different soils are given in Table VII, and plotted in Figs. 4 and 5.   The logarithms of y are also plotted against those of c, giving straight lines, showing that the equation holds both for rich and for poor soils.   In the rich Hoos $2C$ soil (Fig. 5) the experimental numbers all lie on the same side of the bend so that the curves cannot be verified as completely as we would like, but in the poorer soils there is no difficulty on this score (Fig. 4).

TABLE VII.   *Values for y and C for different soils treated with $N/10$ or $N/5$ acid containing varying quantities of Na_2HPO_4.*

Agdell soil.   Time   1 hour. $N/10\ HNO_3$.   Temperature 23° C.

100 gms. of soil to 1000 c.c. of solution.

P_2O_5 brought out by diffusion - 0·025 % - 25 mgms. per 100 gms. of soil.

P_2O_5 added (mgms per 1000 c c)										
P_2O_5 added (mgms per 1000 c c)	0	5 7	11·4	17·1	22 9	28·6	34·3	45·7	57·2	68·6
P_2O_5 found in solution (C) (mgms per 1000 cc)	5·2	6·6	8·9	12 3	15 4	18 1	21·5	29·0	37 2	44·5
P_2O_5 left in soil (y) (mgms per 100 gms)	19·8	24·1	27·6	29·8	32·4	35·5	37·8	41·7	45·0	49·1

Equation obtained : $y - 11·3\ C^{2·6}$

<p align="center">TABLE VII (*continued*).</p>

Saxmundham.   Plot 6 (*unmanured*).

$Time = 2$ *hours.* $N/10$ HNO_3.   *Temperature* 23° C.

<p align="center">50 gms. of soil to 1000 c.c. of solution.</p>

P_2O_5 brought out by diffusion = 17 mgms. per 100 gms. of soil.

P₂O₅ added (mgms. per 1000 c c)	0	6 6	11 0	16 6	22 1	27 6	38 6	49 7	55·2	66 2
P₂O₅ found in solution (C) (mgms. per 1000 c.c.) ...	2 9	6 7	9 8	14 1	18 3	20 8	29 5	39·2	42·6	51·8
P₂O₅ left in soil (y) (mgms per 100 gms)	10 9	17 2	19 9	22 3	25 0	31 1	35 6	38 4	42·6	46·2

<p align="center">Equation obtained: $y - 6 \; 3 \; C^{\frac{1}{2 \cdot 0}}$</p>

Saxmundham.   Plot 1 (*superphosphate*).

$Time = 2$ *hours.* $N/10$ HNO_3.   *Temperature* 23° C.

<p align="center">50 gms. of soil to 1000 c.c. of solution</p>

P_2O_5 brought out by diffusion - 26 mgms per 100 gms of soil.

P₂O₅ added (mgms per 1000 c.c)	0	5 5	11 0	16 5	22 1	27 6	33 1	38 6	55·2	66 2
P₂O₅ found in solution (C) (mgms per 1000 c c) . .	5 6	9 7	13 6	17 3	22 0	27 1	31 6	37 2	51 8	61 1
P₂O₅ left in soil (y) (mgms per 100 gms) . .	14 8	17 6	20 9	24 5	26 0	27 5	29 1	28 9	32 7	36 3

<p align="center">Equation obtained: $y - 7 \; 9 \; C^{\frac{1}{2 \cdot 7}}$.</p>

Agdell soil. $N/10$ HNO_3. $Time = 0$.

<p align="center">100 gms. of soil to 1000 c.c. of solution.</p>

P_2O_5 brought out by diffusion - 25 mgms. per 100 gms. of soil.

P₂O₅ added (mgms per 1000 c.c.)	0	11 4	22 9	34 3	45 7
P₂O₅ found in solution (C) (mgms. per 1000 c.c.) . ..	5 0	12 7	19 1	27 4	36 1
P₂O₅ left in soil (y) (mgms per 100 gms.)	20 0	23 8	28 8	31 9	34 7

<p align="center">Equation obtained: $y = 11 \cdot 5 \; C^{\frac{1}{3 \cdot 1}}$.</p>

TABLE VII (continued)..

$N/10\ HNO_3$. $Time = 24\ hours$.

P$_2$O$_5$ added (mgms. per 1000 c.c)	0	22·9	34·3	45·7
P$_2$O$_5$ found in solution (C) (mgms. per 1000 c.c.)	3·6	7·0	11·2	13·7
P$_2$O$_5$ left in soil (y) (mgms. per 100 gms.)	21·4	40·9	48·1	57·0

Equation obtained: $y = 9\cdot4\ C^{1\cdot5}$.

Hoos 2C soil. $N/10\ HNO_3$. $Time = 1\ hour$.   *Temperature* 23° C.

50 gms. of soil to 1000 c.c. of solution.

P$_2$O$_5$ brought out by diffusion   110 mgms. per 100 gms. of soil.

P$_2$O$_5$ added (mgms. per 1000 c.c.)	0	5·5	10·9	21·8	43·7	65·5
P$_2$O$_5$ found in solution (C) (mgms. per 1000 c.c.)	29·6	34·3	38·3	47·6	66·5	84·1
P$_2$O$_5$ left in soil (y) (mgms. per 100 gms)	50·7	52·4	54·7	58·4	62·7	70·8

Equation obtained: $y = 19\cdot7\ C^{3\cdot6}$.

$Time = 24\ hours$. $N/10\ HNO_3$.

P$_2$O$_5$ added (mgms per 1000 c.c)	0	10·6	21·1	31·6	42·1
P$_2$O$_5$ found in solution (C) (mgms per 1000 c.c.)	26·8	33·3	42·2	48·4	57·0
P$_2$O$_5$ left in soil (y) (mgms per 100 gms.)	56·4	64·7	67·8	76·6	80·3

Equation obtained: $y = 12\cdot0\ C^{2\cdot1}$.

Woburn wheat (unmanured).

$Time = 1\ hour$. $N/10\ HNO_3$.   *Temperature* 23° C.

100 gms. of soil to 1000 c.c. of solution.

P$_2$O$_5$ brought out by diffusion   39 mgms. per 100 gms. of soil.

P$_2$O$_5$ added (mgms per 1000 c.c.)	0	0	5	11·0	16·6	22·1	33·1	44·2	55·2
P$_2$O$_5$ found in solution (C) (mgms. per 1000 c.c)	2·7	3·2	6·2	8·8	12·6	16·1	23·6	32·8	40·3
P$_2$O$_5$ left in soil (y) (mgms. per 100 gms.)	36·3	35·8	38·3	41·2	43·0	45·0	48·5	50·4	53·9

Equation obtained: $y = 30\cdot7\ C^{6\cdot2}$.

TABLE VII (continued).

Bramford subsoil (extracted).*

Time = 1 hour.   *$N/10$ HNO_3.*   *Temperature 23° C.*

100 grms of soil to 1000 c c of solution

P_2O_5 by diffusion – 90·9 mgms. per 100 gms. of soil.
P_2O_5 removed   83·0 ,,   ,,   ,,   ,,
P_2O_5 left in soil   7·9 ,,   ,,   ,,   ,,

P_2O_5 added (mgms per 1000 c c)	0	5 5	10 9	16 4	21 9	27 3	32 8
P_2O_5 found in solution (C) (mgms per 1000 c c)	3 5	7 4	11 7	16 5	21 0	25 2	30 6
P_2O_5 left in soil (y) (mgms per 100 gms)	4 4	5 9	7 1	7 8	8 8	10 0	10 1

Equation obtained $y = 2\,7\,C^{\frac{1}{2\,6}}$

* The Bramford soil contained so much P_2O_5 that it hardly showed the ordinary adsorption phenomena instead therefore of working with the surface soil we used the subsoil, and treated it with $N/5$ HNO_3 to remove part of the P_2O_5

Time = 1 hour   Hoos 2 C. $N/5$ HNO_3

P_2O_5 added (mgms per 1000 c c)	19 0	28 5	38 0
P_2O_5 found in solution (C) (mgms per 1000 c c)	36 2	55 0	63 7	72 6
P_2O_5 left in soil (y) (mgms per 100 gms)	37 6	37 9	39 5	10 8

Equation obtained $y = 18\,7\,C^{\frac{1}{5\,7}}$

Time = 24 hours $N/5$ HNO_3

P_2O_5 added (mgms per 1000 c c)	9 5	19 0	28 5	38 0
P_2O_5 found in solution (C) (mgms per 1000 c c)	48 1	57 1	66 4	73 8
P_2O_5 left in soil (y) (mgms per 100 gms)	31 1	32 7	33 7	36 1	38 4

Equation obtained $y = 10\,4\,C^{\frac{1}{3\,4}}$.

TABLE VII (*continued*)

Time = 1 *hour.*   N/10 *citric acid.*

P_2O_5 added (mgms. per 1000 c.c.)	0	10 5	21·0	31 5	42 0
P_2O_5 found in solution (C) (mgms. per 1000 c.c.) ...	36 1	46·7	55·0	65 8	75 6
P_2O_5 left in soil (y) (mgms. per 100 gms.)	37 8	37 8	42 1	41·5	44·7

Equation obtained: $y - 13 \cdot 4\,C^{3 \cdot 6}$.

Time = 24 *hours.*   N/10 *citric acid.*

P_2O_5 added (mgms. per 1000 c.c.)	0	10 5	21·0	31·5	42 0
P_2O_5 found in solution (C) (mgms. per 1000 c c) ...	43·4	52 4	62 2	71·1	81·3
P_2O_5 left in soil (y) (mgms per 100 gms)	23 2	26·1	27·5	30 8	31·3

Equation obtained: $y = 4 \cdot 1\,C^{2 \cdot 2}$.

It is thus clear that the reverse reaction, *i.e.* the removal of the P_2O_5 from the solution by the soil is an ordinary adsorption, and conforms to the general law expressed by the equation already given.

Turning now to the direct reaction, this must obviously be studied by eliminating the reverse reaction, which as we have seen can be done by the diffusion method. The results for citric, nitric and hydrochloric acids are given in Table VIII and are remarkably alike: considerably more so than when the experiment is carried out by the usual extraction method. Sulphuric acid, however, brings out more P_2O_5 than any of these.

Reference to Table III and Fig. 3 shows that the *net* solvent action of these three acids is very different, citric acid dissolving most, hydrochloric acid next, and nitric acid least.

It follows, therefore, that the reverse reaction, *i.e.* the adsorption of P_2O_5 from the solution is greatest in presence of nitric acid, next of hydrochloric, and least in presence of citric acid.

TABLE VIII. *Quantities of P_2O_5 brought out by diffusion, in comparison with the amounts brought out by ordinary extraction methods. Results calculated as percentage of the soil.*

Soil, Hoos 2C.

	N/10 citric acid	N/10 HNO₃	N/10 HCl	N/10 H₂SO₄	N/10 ammonium citrate
Diffusion	·109	·108	·111	·146	—
Ordinary extraction, 24 hrs.	·084	·054	·045	·077	—

Soil, Agdell.

	N/10 citric acid	N/10 HNO₃	N/10 HCl	N/10 H₂SO₄	N/10 ammonium citrate
Diffusion	·031	—	·032	—	·031
Ordinary extraction, 24 hrs.	·0104	—	·0017	—	·013

Soil, Woburn Stackyard Field, unmanured wheat.

	N/10 citric acid	N/10 HNO₃	N/10 H₂SO₄
Diffusion	·041	·037	·048
Ordinary extraction	·013	·004	·016

The striking result brought out by this experiment is that *the direct solvent action of dilute citric, hydrochloric, and nitric acids on the soil phosphates is the same for all three acids.*

The characteristics of the adsorption.

The fixation of P_2O_5 in soil has been variously attributed to calcium carbonate, to oxides of iron or aluminium, or to humus. None of these, however, is the potent agent in the soils used in our experiments. The fact that adsorption goes on in the presence of acids rules out the carbonates and oxides: the Agdell *B* experiments (Table V, Fig. 3) rule out soluble humus, because this material has already been removed. Subsoils show the phenomena just as freely as surface soils. Moreover extraction of the soil with toluene has no measurable effect on its adsorbing powers; nor does heating the soil till it is charred cause

them to go entirely: not till the soil is ignited is the property lost altogether, and the whole of the added P_2O_5 recovered:

	Ignited soil		Charred soil
	Agdell Surface	Rothamsted Deep subsoil	Rothamsted Garden soil
P_2O_5 obtainable from soil, mgms....	12·2	7·4	83·7
P_2O_5 added, mgms.	10·0	10·0	10·0
Total..............	22·2	17·4	93·7
P_2O_5 found by HNO_3 extraction ...	22·3	16·1	86·6
P_2O_5 adsorbed, mgms...................	Nil	1·3	7·1 mgms. P_2O_5
Percentage	Nil	12 8	71 per 100 gms. of soil

The acids adsorbed. Although we have confined ourselves hitherto to P_2O_5 we have evidence that the phenomena are general and that other acids are adsorbed in the same manner, though in varying degrees. Thus oxalic acid is adsorbed in the presence of nitric acid, giving a curve of exactly the same nature as that for phosphoric acid. (See p. 124.)

Citric acid is also adsorbed but the analytical difficulties proved so considerable that we have been unable to obtain sufficiently reliable results to allow of the drawing of a curve.

On the other hand, we could find no evidence that either hydrochloric or nitric acid is adsorbed to any notable extent.

Thus, it appears that the acids most diminishing the adsorption of phosphoric acid, viz citric and oxalic acids, are themselves freely adsorbed: while the acids which do not so greatly diminish the adsorption, viz. hydrochloric and nitric acids are not adsorbed.

We have examined various possibilities, but the simplest and most in accordance with the facts is that citric and oxalic acids satisfy the adsorption capacity of the soil better than P_2O_5 and thus displace some of it into the solution, while dilute hydrochloric and nitric acids will not, so that much of the P_2O_5 still remains adsorbed. We therefore enquired whether the addition to nitric or hydrochloric acids of substances known to be adsorbed by the soil would increase the net solvent action of these acids by displacing the adsorbed P_2O_5 The results of numerous trials are recorded in Table IX. None of them was successful.

TABLE IX. *Effect of added substances on the reaction between dilute acids and soil phosphorus compounds.*

P_2O_5 extracted in mgms. per 100 gms. of soil.

I. Organic substances: phenol, o-cresol, and pyrogallic acid.

Soil, Hoos 2*C. Time* = 0.

N/10 HNO₃ alone	N/10 HNO₃ + N/10 phenol	N/10 HNO₃ + N/10 cresol	N/10 HNO₃ + N/100 cresol	N/10 phenol alone	N/10 cresol
57	49	57	49	15	8·4

Soil, Hoos 2*C. Time* = $5\frac{1}{2}$ *hours.*

N/10 HCl alone	N/10 HCl + N/10 phenol	N/10 HCl + N/100 phenol	N/10 phenol alone	N/100 phenol alone
63	55	54	8	9·6

Soil, Hoos 2*C. Time* = 0.

N/10 HCl alone	N/10 HCl + N/10 pyrogallic acid	N/10 HCl + N/100 pyro-gallic acid	N/10 pyrogallic acid alone	N/100 pyro-gallic acid alone	Distilled water
48	51	48	17	10	7

II. Inorganic substances: potash-alum, Al_2O_3.

Soil, Hoos 2*C. Time* = 1 *hour.*

N/10 HNO₃ alone	N/10 HNO₃ + potash-alum (0·66 gms. Al₂O₃)	N/10 HNO₃ + Al₂O₃ (0·29 gms.
50	78	26·2

Soil, Agdell. Time = 1 *hour.*

N/10 HNO₃ alone	N/10 HNO₃ + potash-alum (0·66 gms. Al₂O₃)	N/10 HNO₃ + Al₂O₃ (0·29 gms.)	N/10 HNO₃ + Al₂O₃ (0·58 gms.)	N/10 citric acid alone	N/10 citric acid + Al₂O₃ (0·29 gms.)
4·32	11·3	2·28	2·82	7·68	3·42

1000 c.c. N/10 HNO₃, the quantity used in the experiments, is equivalent to 1·7 gms. of Al_2O_3. The extracts made with N/10 HNO₃ and Al_2O_3 were very turbid.

TABLE IX (*continued*)

Soil, Agdell. Time = 3 hours.

N/10 HNO₃ alone	N/10 HNO₃ + 10 c c toluene	N/10 HNO₃ + NH₄NO₃*	N/10 HNO₃ + ammonium oxalate	N/10 ammonium oxalate alone	N/10 oxalic acid alone
7 7	6 7	8 2	24 7	7 9	29 8

N/10 HNO₃ + N/10 amm citrat	N/10 ammonium citrat · alone	N/10 citric acid alone	N 10 HNO₃ + N/10 NaF	N/10 NaF alone	N/5 HNO₃ alone	N/5 HNO₃ + 10 c c toluene
9 6	11 5	10 4	11 0	7 1	9 4	8 2

* Sufficient to make the NO₃ radicle up to N concentration

Phenol, cresol, toluene and alumina, so far from increasing, actually reduce the amount of P_2O_5 brought out by nitric acid, and therefore check the direct solvent action or increase the adsorption. The following experiment shows that the adsorption is increased. 50 gms of the soil Agdell *A*, which had been extracted with dilute sulphuric acid to remove most of the soluble phosphate, was treated with 1 litre of dilute HCl (·06 N) containing 25 % of ethyl alcohol, and a known amount of sodium phosphate was added The following amounts of P_2O_5 were found: they are calculated as mgms per 100 gms. of soil

P_2O_5 added to solution	P_2O_5 found	P_2O_5 adsorbed
9 7	4 2	2 5 without alcohol
9 7	2 5	7 2 with alcohol

All substances do not behave like this however: Table X shows that pyrogallic acid is without effect, and that potash-alum and ammonium oxalate considerably increase the solvent action. These latter substances, however, give rise to sulphuric acid and oxalic acid respectively, both of which have greater net solvent power than nitric acid. In reducing the adsorption of P_2O_5 it is obvious that our only hope would be to light on something which is adsorbed in preference to P_2O_5, and for the moment we have nothing but chance to guide us in the search.

The change of adsorption with the time.

Reverting to Figs. 4 and 5, it is clear that adsorption in presence of nitric acid becomes more pronounced as the length of the action proceeds. Three times were studied: Time = 0, 1 hour, and 24 hours. The adsorption successively increases, and has become very marked after 24 hours.

Agdell Soil.
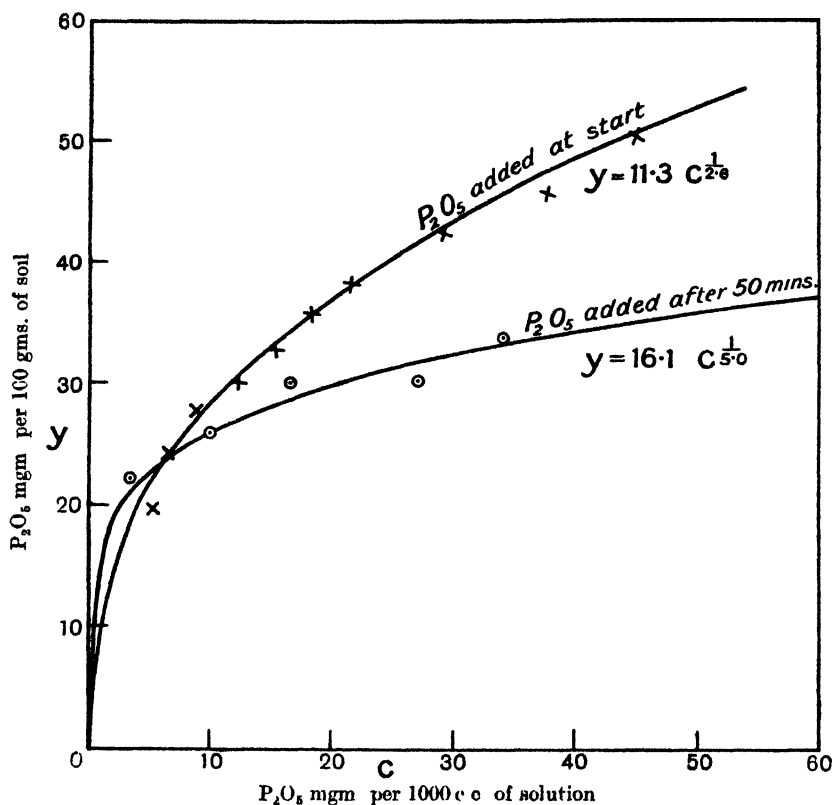
Fig. 9. Effect of composition of the solution on adsorption phenomena.

The first explanation that occurs is that adsorption is a slow business and takes a long while to complete itself. Some support is given to this view by the following experiment:

One lot of soil was shaken for an hour with N/10 nitric acid and sodium phosphate in the usual way. Another lot was shaken with the acid for only 50 minutes, then the phosphate was added and the

shaking done for 10 minutes so as to complete the hour.   The adsorption curves were quite different (Fig. 9).

P₂O₅ added, mgms	0	10·50	21·00	31·50	42·00.
P₂O₅ found in 1000 c.c. solution (C), mgms.	3·2	10·0	16·6	26·8	35·5
P₂O₅ left in 100 gms. soil (y), mgms.	21 8	25·5	29 4	29·7	33·5

Equation obtained: $y - 16 \cdot 1\, C^{\frac{1}{5 \cdot 0}}$

This compares with the experiment recorded in Table VI, Fig. 4a, where the equation obtained is

$$y = 11 \cdot 3\, C^{\frac{1}{2 \cdot 6}}.$$

The experiment is not wholly free from criticism because we do not know exactly what other changes are produced by the acid, or how they are affected by the presence of the phosphate. Against the view that equilibrium is only slowly attained there seems to us to be an insuperable objection. The close agreement between the experimental and the calculated curves shows that a definite equilibrium is attained at each time. The Time 0 curve, for example, indicates just as typical an adsorption as the 24 hour curve. Each adsorption is different, but each is complete in itself. We assume, therefore, until we have stronger evidence to the contrary, that equilibrium is attained instantaneously. This is also true of other cases, animal charcoal, etc.

The next possibility is that the adsorptive capacity of the soil may have increased in consequence of the action of the acid. Considerable change does take place: e.g. quantities of silica are dissolved, and it is possible that the total result of the action may be to increase the adsorptive capacity of the soil. Direct experiment, however, does not support this view. Some of the Agdell soil was treated for 24 hours with N/10 HNO₃, then filtered off, washed and dried. It was then treated for Time 0 with HNO₃, and varying quantities of sodium phosphate. The results are as follows:

Without preliminary treatment

P₂O₅ added, mgms.	0	11·4·	22·9	34·3	45·7
P₂O₅ found in solution (C), mgms	5·0	12·7	19·1	27·4	36·1
P₂O₅ left in soil (y), mgms..........	20·0	23·8	28 8	31·9	34·7

Equation obtained: $y = 11 \cdot 5\, C^{\frac{1}{3 \cdot 1}}$.

	After 24 hours' treatment with N/10 HNO$_3$				
P$_2$O$_5$ added, mgms	0	11·4	22·9	34·3	45·7
P$_2$O$_5$ found in solution (C), mgms	3 1	10·9	17·2	26·5	34·6
P$_2$O$_5$ left in soil (y), mgms	18·3	21·9	27·1	29·2	32·2

Equation obtained: $y = 11 \cdot 5 C^{3\,1}$.

TABLE X. *Effect of alteration of composition of extraction liquid on the adsorption equilibrium in soils. Hoos 2C.*

I. 50·2 gms. of soil treated with 1000 c.c. of an extract obtained by treating soil with N/10 HNO$_3$ for 24 hours and filtering off. 4 c.c. of phosphate solution was also added. Time = 0.

In extract after 24 hours there were 22·08 mgms. P$_2$O$_5$ per litre.

In soil there were 110 mgms. per 100 gms.

Acidity of original acid = 0·0956 equivs. HNO$_3$ per litre.

,, 24 hours' extract - 0·0760 ,, ,, ,,

,, final extract - 0·0660 ,, ,, ,,

P$_2$O$_5$ added	0	11 2	33 5	44 6
P$_2$O$_5$ in solution (1000 c c) (C') ...	31 4	41 9	60 2	67·1
P$_2$O$_5$ in soil (100 gms.) (y)	91·3	92 5	100 5	108 6

Equation is $y = 44 \cdot 1 \, C^{4\,9}$.

II. Fresh acid. 50 gms. of soil to 1000 c.c. of 0·076 N HNO$_3$. Time = 0. In soil 110 mgms. P$_2$O$_5$ per 100 gms.

P$_2$O$_5$ added	0	5 5	16 4	21 8	27 3
P$_2$O$_5$ in solution (1000 c c) (C') ..	23 6	27 1	36 4	41 3	45 6
P$_2$O$_5$ in soil (100 gms) (y)	62 7	66 7	69 9	71 0	73 4

Equation is $y - 34\,5 \, C^{5\,1}$.

III. Fresh acid. 50 gms. soil to 1000 c.c. N/10 HNO$_3$. In soil 110 mgms. P$_2$O$_5$ per 100 gms.

P$_2$O$_5$ added	0	11·2	22 3	33·5	55 8
P$_2$O$_5$ in solution (1000 c c.) (C) ...	19 9	29·4	39·9	49 5	70 0
P$_2$O$_5$ in soil (100 gms) (y)	70 2	73·5	74 8	78·0	81 6

Equation is $y = 50 \cdot 7 \, C^{3 \cdot 6}$.

These are plotted in Fig. 6, and the two curves superpose. The 24 hours' treatment then, much as it altered the soil, did not alter its adsorptive capacity.

There remains the possibility that the solution obtained at the end of 24 hours affects the equilibrium differently from that obtained after Time 0. There is considerable *à priori* justification for such a view, inasmuch as the addition of alumina to the nitric acid depresses the amount of P_2O_5 extracted, and may therefore very well increase the amount of adsorption.

This was tested by treating two lots of the same soil (*a*) with N/10 HNO_3 that had already been in contact with the soil for 24 hours, and (*b*) with fresh HNO_3 of equivalent concentration. If the cause of the difference of adsorption lay in the changed condition of the liquid then adsorption from the 24 hours' liquid should be greater than that from the fresh acid.

Experiment showed that this actually happened. The curve obtained with fresh acid lay below that obtained with the 24 hours' extract, the two being related in the same way as those for Time – 0 and for Time – 24 hours (Table X, Fig. 7).

CONCLUSIONS.

The results enable us to explain what happens when a soil is shaken with a dilute acid in the ordinary process of soil analysis.

The acid dissolves out such phosphorus compounds as it can, and different acids have much the same effect at equivalent concentrations: nitric, hydrochloric and citric acids give the same results; sulphuric acid, however, gives a somewhat higher result.

A reverse reaction at once sets in, however. Some of the P_2O_5 is withdrawn from the solution in spite of the presence of excess of acid. The process is an ordinary adsorption and obeys the usual law expressed by the equation $y = Kc^{\frac{1}{p}}$. Its extent varies with the different acids; it is much more marked in the presence of nitric than of citric acid.

The amount of P_2O_5 actually determined by the analyst is, therefore, not the true amount dissolved, but the difference between these two wholly distinct actions.

It is now obvious why the amounts of "available P_2O_5" determined by extraction with dilute acids shows such great variations in different methods of analysis, and so little correlation with the actual quantities

obtainable by the crop. In no case do they stand for anything actual, but only for a difference between a direct action and an adsorption which varies with the nature of the acid and the conditions of the experiment.

So long as they are confined to the same type of soil, however, any of the acids investigated would have given useful results, but difficulties would arise directly an attempt was made to compare dissimilar soils. The proper way to use a soil analysis is in conjunction with a soil survey.

A diffusion method is described in which the reverse reaction is eliminated, and which therefore gives a true measure of the direct action. But until we have had more experience with it we are not prepared to say what value it has for soil analysis.

THE PHENOMENON OF ABSORPTION IN ITS RELATION TO SOILS.

A RÉSUMÉ OF THE SUBJECT.

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The facts of Absorption.

It has been known from the earliest times that soils would remove salts and colouring matters from solution and the problems arising out of the observations made on this phenomenon were among the earliest to be attacked by agricultural chemists. It was known to Aristotle that sea water lost some of its taste by filtration through sand and this observation seems to have been confirmed and applied in many ways. Lord Bacon in his *Sylva Sylvarum*[1] discussed the question of making sea water potable by filtering through sand. Le Comte de Marsilli[2] made quantitative experiments. Sea water was filtered through fifteen successive vessels of garden soil and a diminution in the salt content was proved by evaporation and by the change in specific gravity. Similar results were obtained with sand. Boyle Godfrey[3] discussed the question of making sea water fit for use on ships and observed that if sea water be put into a stone straining cistern the first pint that runs through will be like pure water, having no taste of salt, but the next pint will be as salt as usual. Stephen Hales[4] in dealing with the same question refers to the use of a soft stone by the Dutch as a filtering material, but he points out that this method has no practical value as only the first portions of the filtrate are free from salt.

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[1] §§ 1 and 882.
[2] *Histoire physique de la Mer,* 1725
[3] *Miscellaneous Experiments and Observations,* 1737.
[4] *Philosophical Experiments,* 1739.

A more definitely agricultural observation was made by Gazzeri[1] who observed that soil and especially clay take up soluble substances which he considered an advantage inasmuch as they may become available later as the plants need them.

Lambruschini[2] suggests a special kind of combination (*incorporamento*) between plant nutrients and soil which was neither so weak as to allow them to be washed out nor yet so strong as to interfere with their absorption by the plant.

J. P. Bronner[3] observed that when river sand was shaken in a bottle with liquid manure both the smell and the colour were largely removed. This was confirmed independently by Huxtable in 1848. Liquid manure filtered through a loamy soil lost smell and colour[4], "it went in manure and came out water."

The first quantitative experiments were made by H. S. Thompson in 1845[5]. Soils were mixed with a solution of ammonium sulphate and washed through with water. Analysis showed that considerable quantities of ammonia had disappeared from the solution while calcium and magnesium sulphates were present. The sulphuric acid radical was not absorbed.

Way's chemical hypothesis.

J. T. Way followed up this work[6] and extended it to other bases: he showed that ammonia was absorbed from all its salts, so also were potassium, sodium and magnesium from their salts. Lime was also found to be absorbed from caustic lime and from bicarbonate solutions, but other salts were not tried because Way assumed that it was useless, there being no provision in the soil for the decomposition that would be first necessary. Burning a soil was found to diminish the absorption by about one half and thoroughly ignited clays showed no absorption whatever. Absorption both of ammonia and potash increased with the strength of the solution but not proportionately. Way supposed the phenomena to be chemical. Matteucci[7] had already explained the absorption of salts by sand by assuming that the particles of sand have a greater attraction for salts than they have for water,

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[1] 1819 *Text Book of Manuring*, quoted by A. Orth, *Landw. Versuchs-Stat.* 1873, **16**, 56.

[2] *Atti dei Georgofili di Firenze*, 1830, **9**, 330, quoted by F. Sestini, *Landw. Versuchs-Stat* 1873, **16**, 409.

[3] *Der Weinbau in Süddeutschland*, 1836.   [4] Quoted by Way.

[5] *Journ. Roy. Ag. Soc.* 1850, **11**, 68   [6] *Ibid.* 313

[7] *Sur les phénomènes physiques des corps vivants*, 1847.

absorption being thus a manifestation of capillarity. Way pointed out that this physical action deals with the salt as a whole · in the type of soil absorption with which he was working, bases only were affected. This pointed to a chemical action. Again, while physically absorbed substances could be washed out with water, the chemically absorbed substances were completely insoluble.

In a following paper[1] Way develops the idea of absorption by precipitation of insoluble compounds in the soil. He assumed the presence in the soil of a small proportion of double silicates of lime and aluminium which by reaction with salts of ammonia or potassium gave rise to insoluble ammonium or potassium aluminium silicates and the corresponding calcium salts. He prepared double silicates by mixing solutions of alum and sodium silicate- -a double silicate of sodium and aluminium was precipitated which by treatment with excess of calcium chloride gave the corresponding calcium compound.

The behaviour of this calcium compound towards salts was in every respect similar to that of soils and Way had "every certainty" that these double decompositions were "the efficient cause of the singular power."

These experiments were subsequently confirmed by A. Voelcker[2] who attributed the beneficial effect of common salt as a manure to its action in setting free potassium from its combinations in the soil[3].

R. Warington[4] also held that the phenomenon was to be explained on chemical grounds. He found that precipitates of aluminium and iron hydroxides were also efficient absorbents for both phosphates and bases; this he explained as due to the weak chemical affinity. He supposed that to these oxides was due at least to some extent the peculiar properties of soils, but he did not deny the possibility of the presence of Way's double silicates.   •

Liebig's physical hypothesis.

J. v. Liebig[5] criticised Way's hypothesis. He compared the action of the soil with that of wood or animal charcoal, and regarded the attraction of soil for salts as being of a purely physical nature. He divided plant food constituents into two classes, one chemically and the other

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[1] *Journ. Roy. Ag. Soc.* 1852, **13**, 123
[2] *Ibid.* 1860, **21**, 105; 1864, **25**, 333.
[3] *Ibid.* 1865, **26**, 298.
[4] *Trans. Chem. Soc.* 1868, **21**, 1.
[5] *Ann. Chem. Pharm.* 1858, **105**, 109; **106**, 185.

physically combined in the soil, considering the latter only to be that immediately available to plants. "The power of the soil to nourish cultivated plants," he states, "is therefore in exact proportion to the quantity of nutritive substances which it contains in a state of physical saturation. The quantity of other elements in a state of chemical combination distributed through the ground is also highly important, as serving to restore the state of saturation, when the nutritive substances in physical combination have been withdrawn from the soil by a series of crops reaped from it[1]."

Knop's compromise and analytical method.

W. Knop[2] in reviewing the subject attempted to combine Way's chemical and Liebig's physical hypotheses. He explains the removal of bases from solution by surface attraction and by combination with silica or double aluminium silicates. The absorption of phosphates he attributed to the precipitation of calcium phosphates in the first instance and afterwards to the formation of iron and aluminium phosphates. Other acids were said to be held up also by iron and aluminium hydroxides with the formation of basic salts. Knop recognised that the chemical explanation was insufficient to account for the absorption of the bases, because an equilibrium was invariably established between the soil and the solution and the whole of the base could not be removed from its solution no matter how dilute this might be.

The chief facts concerning absorption by soils were accumulated by about 1880. Since then very little has been added except on the theoretical side. W. Henneberg and F. Stohmann[3] treated soils with different ammonium salts in varying amounts, and Peters[4] worked with potassium salts. Both obtained the same results as Way: as the concentration of the salt was increased the amount of base taken up by the soil increased also but was in lower proportion; *i.e.* the relative amount taken up decreased with the increase in concentration of the solution. A vast amount of subsequent work has shown that this is true of all absorption phenomena and the relationship has more recently been expressed mathematically. Two methods of investigation have been used: (1) the soil is brought into equilibrium with a definite

[1] *Natural Laws of Husbandry*, 1863, pp. 67–69.
[2] *Lehrbuch der Agriculturchemie*, Leipzig, 1868.
[3] *Journ. f. Landw.* 1859. **3**. 25.
[4] *Landw. Versuchs-Stat.* 1860, **2**, 113.

volume of solution, and (2) a solution is filtered through a column of soil. The first is by far the more accurate although there are occasions when the second is useful.

The effect of bringing a soil into equilibrium with a solution of potassium or ammonium salts is that some of the potassium or ammonium disappears from the solution, and other bases, chiefly calcium and magnesium, come in, which together are approximately equivalent to the potassium or ammonium absorbed. This was discovered by Way and has since been abundantly confirmed. E. G. Parker[1] states that the total quantities of Ca, Mg and Al in a solution of potassium chloride after treatment with a soil were not quite equivalent to the amount of potassium absorbed but that the difference was practically equivalent to the amount of free acid present in the solution.

The effect of the presence of other salts on the absorption of any particular base was studied by A. Frank[2]. A solution of potassium chloride was caused to percolate through a column of soil, the effluent was drawn off at various depths and the amount of potassium absorbed was determined. Sodium chloride was then added to the potassium chloride solution and a fresh percolation carried through; it was found that the presence of sodium salt lowered the absorption of the potassium which was consequently found in high concentrations even in the lower parts of the soil column.

The absorbed potassium or ammonium was found to be partially removed by a solution of a calcium salt but none of the early workers were able to obtain complete replacement[3].

As pointed out above, Liebig considered that the absorbed bases of a soil were those immediately available to the plant. W. Knop[4] devised a method for determining the available plant food based on this assumption. He put the matter thus: "Soils of great fertility have a high absorptive power": "Soils of great fertility have a high content of easily replaceable bases" and on this account should show high absorption towards salt solutions. He therefore compared a series of 100 soils by determining the absorption of ammonia from a 0·5 % solution of ammonium chloride. The results obtained were in fair agreement with the agricultural history of the soils.

[1] *Journ. Agric. Research.* 1913. **1**, 179
[2] *Landw. Versuchs-Stat.* 1866. **8**, 45.
[3] Way, Eichhorn, Dietrich, Peters, Schuhmacher. References are given elsewhere.
[4] *Bonitirung der Ackererde*, 1871.

E. Biedermann[1] made a further comparison with 23 soils from various parts of Saxony and also obtained results in fair agreement with the assumption—exceptions were noted chiefly where the absorbed ammonia was replaced almost entirely by magnesium, the soils being then somewhat infertile. O. Kellner[2] varied the procedure somewhat, and determined the amount of available bases by boiling the soil with successive quantities of ammonium chloride solution. He states that the amounts of potassium and calcium thus removed are precisely equal to the quantities obtainable by plants. This he showed by the following experiment.

Twenty-two pea plants were grown for six weeks in 369·15 gms. of soil placed in a funnel plugged with asbestos and watered with a diluted solution of ammonium nitrate, 1 gm. of which was supplied during the whole period. The soil was analysed for available plant food by the above method. The amount of food material in the plants was also determined. The results are as follows:

	K_2O	CaO		K_2O	CaO
	gm	gm		gm.	gm.
Found in plants	0 1041	0 0417	In original soil	0·2208	1 1235
Originally in seed	0·0449	0 0060	After experiment	0 1612	1 0887
Removed from soil	0 0592	0·0357	Difference	0 0596	0 0348

This rather remarkable statement does not seem to have been since confirmed in spite of its obvious importance.

Recently E. Ramann has introduced a further modification: a 5 % ammonium nitrate solution is allowed to percolate through the soil and the displaced potassium or other plant food is determined. He speaks of this as a "Base exchange" (*Basenaustausch*)[3]. A similar "Base exchange" was obtained by O. Kullenberg[4] with calcium, magnesium and sodium salts. Using these in various concentrations the final equilibrium resembles those already attained by Peters with potassium and Henneberg and Stohmann with ammonium salts.

[1] *Landw. Versuchs-Stat.* 1869, **11**, 1; 1872, **15**, 21.

[2] *Ibid.* 1886, **33**, 349 and 359.

[3] J. A. Hankey, *Nature*, 1914, **93**, 598. International Commission on the Chemical Analysis of Soils.

[4] *Jahresb. Agric. Chemie*, 1865, **8**, 15.

Absorption of acid radicals.

The absorption of acid radicals has not been much discussed, chiefly because the early workers invariably found as much Cl, SO_4, or NO_3 in all their solutions after as before treatment with a soil. In the case of phosphoric acid the problem has been automatically removed from the discussion because most soils contain sufficient lime to precipitate soluble phosphates chemically. The soil phosphates have therefore been divided up into available or unavailable classes according to their solubility. Recent work however has shown that phosphoric acid can be physically absorbed by a soil in the presence of excess of acids like citric, nitric or hydrochloric acid. This absorption is discussed elsewhere[1]. The absorption of oxalic and citric acids (see p. 124) has been observed by the author to take place in the presence of nitric or hydrochloric acids. U. Pratolongo[2] has shown that there may be two distinct processes—with soils containing no lime, absorption of phosphoric acid from monocalcium phosphate solution is practically instantaneous, with a soil containing much calcium carbonate there is a further slow fixation of phosphoric acid after the initial absorption. The immediate process is regarded as an absorption, the second as precipitation. His results are as follows:

Time	Soil containing no lime	Soil containing 10·3 % of lime
5 mins	307 mgms	774 mgms
30 ,, ...	315 ,,	903 ,,
1 hour	312 ,,	1101 ,.
6 hours.	315 ,,	1774 ,,

The absorption of other acid radicals has not yet been observed in the case of soils.

Effect of Temperature.

Small changes in temperature seem to be without appreciable effect on absorption, but W. Schuhmacher[3] found that at high temperatures the absorption was usually less than at low temperatures.

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[1] See *Proc. Chem. Soc.* 1914, **30**, 123, and this *Journal*, p. 65

[2] *Le Stazioni Sperimentali Agrarie*, 1915, **48**, 457.

[3] *Ann. der Landw.* 1867, **49**, 322.

Absorption by Humus.

The part played by humus in the absorption processes in the soil has not been very fully worked out. J. M. Van Bemmelen[1] showed that humus is a typical colloid possessing absorbing powers similar to those of the gels.

A solution of humus in ammonia is precipitated by the addition of salts of metals such as copper, lead, calcium, magnesium—these so-called humates have been and still are in many quarters assumed to be true insoluble salts. Van Bemmelen showed that their composition was very variable and hence defined them as absorption complexes.

A. König[2] showed that a sphagnum moor soil consisting almost entirely of organic matter absorbed potassium from its salts with substitution of calcium and magnesium. Humus shows its greatest absorptive power with alkalies; its power of taking up ammonia is well known and is shown by the insoluble portion as well as by that soluble in alkalies. Peat which is almost entirely free from calcium and other bases still absorbs phosphoric acid. It also absorbs neutral salts as a whole. König also obtained negative absorption, *i.e.* the absorption of water instead of dissolved salt from the solution. A peat in contact with N/10 KCl solution absorbed water alone and left the solution more concentrated than before

When an alkaline solution of humus is precipitated it carries down with it some of the substances present in the solution. W. Schuhmacher[3] using "humus" obtained by the action of sulphuric acid on sugar found an absorption of from 1·7 to 10 % from solutions of 0·5 % of sodium phosphate, potassium nitrate and calcium chloride. Humus is a very difficult substance to work with as there are so many fractions and so far no extensive attempts have been made to deal systematically with its absorptions.

In the author's experiments the amount of phosphoric acid absorbed by the humus was independent of the concentration of the phosphate solution. A strong solution of humus was made in ammonia the excess of which was then removed by a current of air. The solution was then made slightly acid with acetic acid and dialysed for three days. Considerable quantities of phosphate were removed, and finally the solution contained 15 mgms. of P_2O_5 per 100 c.c. Portions of 25 c.c. of this

[1] *Landw. Versuchs-Stat.* 1888, **35**, 67.
[2] *Landw. Jahrb.* 1882, **11**, 1.
[3] *Ann. d. Landw.* 1867, **49**, 322.

solution were made up to 100 c.c. with dilute nitric acid (making the acidity N/20) and varying amounts of sodium phosphate solution; after three hours' shaking, the precipitates were filtered and P_2O_5 determined in 50 c.c. of the filtrate. The following results were obtained:

Mgms. per 25 c.c humus solution

P_2O_5 added + P_2O_5 in humus (3·75)	P_2O_5 found in solution	P_2O_5 fixed by humus
14·22	11·58	2·64
16·86	14·70	2·16
18·60	16·30	2·30
21 21	19 60	2·60

Much work remains to be done on the quantitative relationships in the absorption by the organic matter of soils.

The introduction of the conception of colloids.

Van Bemmelen was the first to introduce the conception of colloids into soil problems. The work of Thomas Graham (1861–1864) on colloidal solutions had led to the more precise definition of these bodies. He showed that solutions of gelatine, glue. gum arabic would not diffuse freely through organic membranes like parchment, whilst solutions of the ordinary crystalline substances would. He therefore divided substances into "crystalloids" and "colloids." This division is no longer sharply defined, as many substances can be obtained both in the crystalloid condition and in colloidal solution. Graham also found that certain solutions such as silicic acid, tungstic acid, aluminium and iron hydroxides would not diffuse through parchment; he therefore called these: "colloidal solutions" or "sols." These solutions however did not behave like true solutions in that they were radically changed by the addition of small quantities of electrolytes. The "sol" of silicic acid sets to a jelly in the presence of a trace of carbon dioxide, while a trace of sodium sulphate would precipitate a ferric hydroxide or aluminium hydroxide "sol." The precipitates or the jellies were termed "gels." Van Bemmelen investigated their properties, especially the rate at which they lost water by evaporation and their absorptive power for water and solutions. He further showed that these phenomena in gels resembled some of the known reactions of soils. It is interesting

to trace the development of his ideas.   In his first paper[1] he investigated
the relationship between the amount of absorption and the easily
decomposable silicates of the soil.   Here he followed Way's earlier
assumption as to the seat of the absorptive power of the soil and the
later results that many natural "zeolotic" silicates, basalt, natrolite,
etc., showed similar phenomena when brought into contact with salt
solutions (Eichhorn[2], Lemberg[3]).   Heyden[4] had shown that a soil after
treatment with hydrochloric acid gives up silica to a solution of caustic
soda.   Van Bemmelen regarded the amount of silica thus liberated
as an indication of the quantity of easily decomposable silicates present.
He generally found that soils which absorbed well also gave up much
silica.   After a soil had been boiled with hydrochloric acid, it took up
only small quantities of potassium from a solution of neutral potassium
salt but large quantities from the hydroxide or carbonate solutions.
If such a soil were first treated with sodium hydroxide, then it would
react normally with neutral potassium salts, potassium being taken up
and sodium appearing in the solution.   These reactions were attributed
to the silica "gel" present in the soil after boiling with acid.

He concludes that soil absorption is a chemical and not a physical
phenomenon, basing his conclusions chiefly on the fact that an exchange
of bases always takes place.

In subsequent papers his opinions change.   This later work is
chiefly on absorption by simple gels, *e.g.* of silica, tin oxide, iron oxide
and alumina.   He found that neutral salts and acids were absorbed
by silica in small quantities; in this case there could be no possibility
of chemical reaction.   He thus recognises the possibility of physical
absorption of hydroxides and carbonates by silica and where substitu-
tion takes place he says that the substitution is not really chemical
but only apparently so[5].   In 1900[6] he definitely stated that if any
absorbent is in equilibrium with a solution of a substance C_1 and a
second substance C_2 is brought into the solution, then partial sub-
stitution of C_1 by C_2 will take place.   Van Bemmelen thus partially
removed aluminium chloride from a silica gel, sulphuric acid from a
manganese dioxide gel and calcium chloride from a chromium hydroxide
gel by means of potassium chloride and potassium sulphate.

[1] *Ber. Deut. Chem. Ges.* 1878, **11**, 2228; also *Landw. Versuchs Stat* 1878, **23**, 265.

[2] H. Eichhorn, *Landw. Jahrb.* 1875, **4**, 1.

[3] J. Lemberg, *Zeits. d. deutschen geol. Ges* 1883, **35**, 557.

[4] *Ann. d. Landw.* 1864, **43**, 310.

[5] *Die Absorption*, p. 100; *Landw. Versuchs-Stat.* 1888, **35**, 67.

[6] *Ibid* p. 441, *Zeits f. anorg Chemie*, 1900, **23**, 321.

Similarly a silica gel was treated with calcium hydroxide and afterwards with potassium chloride solution. The potassium removed much of the calcium but the chloride radical remained in solution.

He concludes[1] that the phenomena of absorption in soils are of the same nature as in artificial calcium aluminium silicates brought into contact with the solutions of salts of the alkalies. Thus the soil absorptions fall into line with other absorption phenomena.

Adsorption.

Much of the recent work on the absorptive power of soils has been of a physico-chemical nature as it has been recognised that more definite knowledge as to the nature of the phenomena will be obtainable by this means. Before proceeding to the discussion of these developments it will be necessary to deal with the general phenomenon known as "adsorption" This subject has received considerable attention during the last ten years from the physical chemists and more recently from the physicists[2]. "Adsorption" is the concentration of any substance on the surface of another. The power of charcoal to absorb gases and to remove colouring matter from solution is perhaps the most familiar example.

Certain phenomena occur at the surface bounding any two phases of a system which are generally associated with the effect of Surface Tension. In the case of a liquid in contact with a gas this tension is a well defined and measurable constant. The surface energy of any system tends to become a minimum; in the case of a liquid in contact with a gas, the liquid tends to become a perfect sphere, which has the minimum surface and hence the minimum surface energy. Where the bulk of the liquid is too great and the force of gravity prevents the liquid becoming spherical the surface remains practically constant but the surface energy nevertheless falls if the opportunity occurs. The addition of soluble substances to a liquid usually changes the surface tension. If an increase of concentration decreases the surface tension, then a concentration of the solute occurs in the surface layer; in other words adsorption takes place. If however the addition of a solute increases the surface tension, the concentration of the solute in the surface layer diminishes, giving rise to "negative adsorption." These deductions from thermodynamical laws have been confirmed by experiments. Miss C. C. Benson[3] has shown that the froth obtained from a solution

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[1] *Die Absorption*, p. 433.   [2] See Trouton, *Brit. Assn Report*, 1914.
[3] *Journ. Phys. Chem.* 1903, **7**, 532.

of amyl alcohol in water has a higher concentration of the alcohol than the original solution; amyl alcohol lowers the surface tension of water. J. v. Zawidzki[1] has obtained similar results with saponin.

It is extremely probable that the surface liquid-solid also shows surface tension phenomena, but so far no methods have been devised for determining its magnitude; but the above reasoning is still applied. The further discussion of the theoretical side of this subject leads into the realms of molecular physics and lies outside the scope of this paper. Negative absorption, *i.e.* the decrease in concentration at the surface of the solid, was first observed by C. Matteucci[2] who found that the density of a solution of sodium carbonate was increased by filtration through three metres of sand. G. Gore[3] investigated very fully the absorption of various salts by silica and obtained 13 cases of negative absorption, 97 cases with positive adsorption and 30 cases with no result. S. Lagergren[4] found negative adsorption in the cases of sodium, potassium and ammonium chlorides and animal charcoal. A. M. Williams[5] found that the adsorption of potassium chloride and magnesium sulphate by charcoal increases with the concentration to a maximum, then decreases, then passes through zero and finally becomes negative.

F. T. Trouton[6] has shown that a similar maximum of adsorption occurs at a definite concentration with certain sulphates and nitrates in contact with silica. The adsorption is not much influenced by small changes of temperature, but is diminished by a rise in temperature; below the critical concentration as given in the above experiments of Trouton an increase of adsorption follows a rise of temperature.

The adsorption isotherm.

Generally speaking the adsorption increases with the concentration of the solution according to a simple relationship which can be expressed.

$$\frac{Y}{M} = KC^{\frac{1}{P}},$$ where Y = amount of substance adsorbed by M of adsorbent,

C = concentration of solution in equilibrium with Y,

K and P are constants.

[1] *Zeits. phys. Chem.* 1903, **42**, 612.

[2] *Sur les phénomènes physiques des corps vivants*, 1847.

[3] *Birmingham Phil. Soc.* 1893, **9**, 1; see *Chem. News*, 1894, **69**, 22, 33, 43.

[4] *Bihang till K. Sv. Vet. Akad. Handl.* 1898, **24**, Afd. 11, No. 4.

[5] *Trans. Faraday Soc.* 1914, **10**, 155.

[6] *British Assn. Report*, 1914; *Nature*, 1914, **93**, 642.

This equation in various forms has been used for many years and was recognised by Van Bemmelen in 1878 as being applicable to one of his absorption results[1] but was not followed up by him. He only insisted that the ratio of the absorption to the equilibrium concentration was never constant.

It has been found to hold for the absorption of iodine by starch by F. W. Küster[2] and for picric acid and silk by J. Walker and J. R. Appleyard[3]. Freundlich and his co-workers have established the general applicability of this law since 1906[4]; the law holds in most cases of adsorption so far investigated using as solvents, water, alcohol and other organic solvents and as adsorbents, charcoal, silica and alumina, and various textile fibres.

A typical example of an adsorption curve is given in Fig. 1 from experimental data obtained by the author. This shows the adsorption of oxalic acid by a soil, in the presence of $N/20$ HNO_3 to prevent the chemical precipitation of insoluble oxalates. 25 gms. of a deep Rothamsted subsoil were shaken with 1000 c.c. of varying concentrations of oxalic acid in $N/20$ nitric acid in a thermostat at $23°$ C. for one hour. The oxalic acid was determined in the extracts by titration against potassium permanganate solution; the results obtained are given in Table I.

The experimental points are seen to lie fairly well on a curve expressed by the formula

$$\frac{Y}{M} = \cdot 28\, C^{\frac{1}{3\cdot 56}},$$

which is of the usual adsorption type, $\frac{Y}{M} = KC^{\frac{1}{P}}$. The values for P usually lie between 2 and 10: there are some exceptions but in the majority of adsorptions so far investigated these are the limits. The value of K varies of course with the units employed to express the results and is proportional to the active surface of the adsorbent.

Other formulae have been proposed, notably by S. Arrhenius and by G. C. Schmidt, but at the present time the one given above is most generally used and it gives fair agreement so long as the concentrations of the solutions are not too high.

The rapidity with which the equilibrium is reached is very high; under favourable conditions the process may be said to be instantaneous.

[1] See W. Ostwald in editorial to *Die Absorption.*
[2] *Liebig Annalen*, 1894, **283**, 360.
[3] *Trans. Chem. Soc.* 1896, **69**, 1334.   [4] See *Kapillarchemie*, 1906.

G. C. Schmidt[1] has found that adsorption is slowest if the adsorbent is placed directly into the solution. If the equilibrium is first established and the solution is then diluted the new equilibrium is attained almost immediately. Air films in the adsorbent are suggested as a cause for the slower rate with the fresh material.

TABLE I. *Absorption of oxalic acid by soil.*

Oxalic acid		
added	found in solution C	retained by soil $\dfrac{Y}{M}$
gms.	gms per litre	gms. per 25 gms of soil
0 094	0 017	0 077
0 157	0 046	0·111
0 220	0 078	0 143
0 283	0 121	0 163
0 315	0 148	0 166
0 441	0 253	0·188
0 567	0 356	0 211
0·756	0 526	0·230
0 945	0 702	0 243
1 260	0 980	0 280

$$y/m = \cdot 28\,c^{\frac{1}{5}}$$
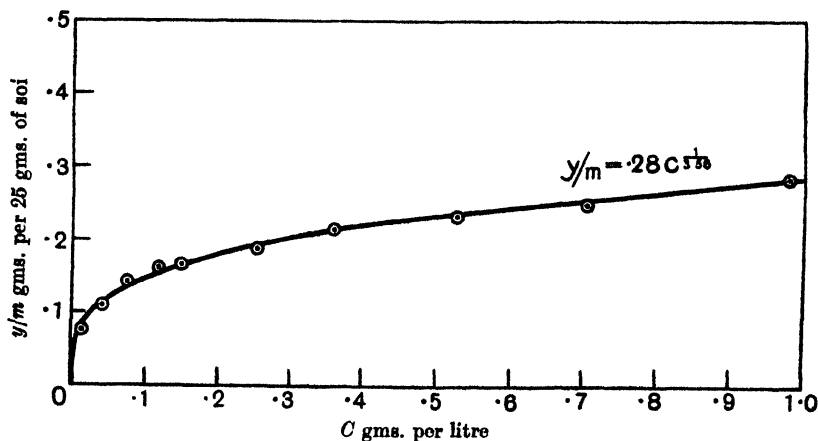
Fig. 1. Absorption of oxalic acid by soil in presence of excess nitric acid.

The simultaneous adsorption of two substances from solution is of much interest to the soil chemist. As already stated Van Bemmelen had shown that one adsorbed substance can be replaced by another

brought into the solution. Adsorption proceeds normally in the presence of a second substance but the constants of the isotherm are changed. Masius[1], Michaelis and Rona[2] and Schmidt have shown that if two substances are adsorbed the adsorption of each one is diminished but it can still be expressed by the usual equation.

Van Bemmelen discusses the question whether absorption by gels is of the same nature as ordinary adsorption by charcoal, silica, etc. A web structure is attributed to the gelatinous precipitates and he points out that there is no essential difference in these cases between adsorption and absorption but he prefers to use the term " absorption" as being more general. In support of this view those precipitates which were supposed to have the largest "web" surface showed the highest adsorptive power and also those precipitates which after ignition were capable of becoming incandescent also showed very active adsorption. The gels used by Van Bemmelen must not be regarded as jellies in the usually accepted sense of the term. One of his silica gels is described as "a dry dusty powder" and most of his absorbents must have been in this form. They can only be described as colloids in that they possessed a structure in which the discontinuities were of colloidal dimensions, that is between $0.1\,\mu$ and $1\,\mu\mu$. Adsorption is not a property peculiar to colloids although colloids show this property to a remarkable extent on account of the large surface they possess in proportion to the total mass.

Applications to soils.

Side by side with all this work, agricultural chemists have been trying to apply the above laws to the known phenomena of absorption by soils. The simplest type of absorption by a soil is shown by a dye-stuff. Here there is no chemical reaction but every probability that the phenomenon is purely physical. The adsorption of dyes by soils is found to follow the usual laws. H. E. Patten and F. K. Cameron[3] found a definite equilibrium with gentian violet, eosin and a manure extract which, when plotted as a curve, was seen to be of the usual adsorption type. Although they recognised the value of the adsorption equation for more simple cases, they did not attempt to express their soil curves by this equation as they considered the soil conditions were too complex. They concluded however that the distribution of solute

[1] *Dissertation*, Leipzig, 1908
[3] *Journ. of Phys. Chemistry*, 1907, **11**, 581.
[4] *Biochem Zeits.* 1908, **15**, 196.

between solvent and absorbent presents in general the same characteristics with soil as with other absorbents.

The difference between the absorption of a base from a neutral salt solution and of a dyestuff is that in the first case an equivalent of other bases is always found in the solution after equilibrium is established. This at first sight points to a chemical reaction in which two insoluble bodies are concerned.

As early as 1859 C. Boedeker[1] setting out from Henneberg and Stohmann's results formulated a mathematical expression for the relationship between the ammonia absorbed by a soil and the calcium turned out, which is substantially that obtained nearly 50 years afterwards by A. D. Hall and C. T. Gimingham[2]. If we call the calcium complex in soil $Ca(X)_2$, then on treating with ammonium chloride the following reaction takes place:

$$2NH_4Cl + CaX_2 \rightleftharpoons 2(NH_4)X + CaCl_2,$$
$$\quad a \qquad b \qquad\qquad c \qquad d$$
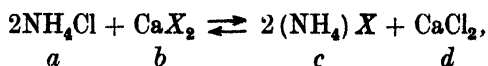
where a, b, c, d are the respective concentrations of the reacting substances, then $\dfrac{a^2}{d} = K$, the active masses of the insoluble substances being constant.

Hall and Gimingham found this law to hold with fair accuracy for the reaction of china clay with ammonium chloride and they concluded that a simple chemical reaction was a sufficient explanation of the results obtained.

Adolf Mayer[3] had pointed out that Boedeker's equation does not apply to the potassium results of Peters. Moreover the active mass of the calcium represents also the amount of ammonia absorbed by the clay, and the equation $\dfrac{a^2}{d} = K$ therefore becomes a special case of the usual adsorption isotherm where $P = 2$.

Attempts to explain the equilibrium results on physical lines have been made by Patten, Cameron and Parker in the United States and by Wiegner in Germany. Patten and Cameron in the paper quoted above worked out Peters' potassium results and found them to be of an adsorption type. E. G. Parker[4] working with potassium chloride and J. H. Aberson[5] working with ammonium chloride and soils obtained similar results.

[1] *Journ. f. Landw.* 1859, 48.
[2] *Trans. Chem. Soc.* 1907, **91**, 677.
[3] *Lehrbuch der Agriculturchemie,* 1886.
[4] *J. Agric. Research*, 1913, **1**, 179.
[5] *Kolloid Zeits.* 1912, **10**, 13.

G. Wiegner has recently reviewed the whole subject[1] and shown that the absorption of ammonia by permutite (a technical calcium aluminium silicate) from a solution of ammonium chloride follows the absorption laws very completely; the equilibrium is instantaneous when reached from above and the absorptive power is dependent largely on the physical condition of the permutite. The Cl ion is not absorbed. The equilibrium attained was expressed by the formula

$$\frac{Y}{M} = 3 \cdot 429 \, C^{0 \cdot 398}.$$

The following table is worked out by Wiegner from the results of various experiments and shows that the adsorption isotherm for soils is of the usual type:

Absorbent			Solute	K	$\dfrac{1}{P}$		Worker
Garden soil	...	...	NH$_4$Cl	0·0948	0·039	}	Henneberg and
,, ,,	...	...	NH$_4$Cl	0·131	0·424	}	Stohmann
Nile sediment	...	...	NH$_4$Cl	0·489	0·399		Armsby
Permutite	}		NH$_4$Cl	2·823	0·398		Wiegner
Sodium zeolite	} artificial		CaCl$_2$	2·487	0 317		Armsby
Zeolite	}		LiCl	24·419	0·414		Campbell
Soil	...	...	NH$_4$OH	0·0994	0·434	}	
,,	..	...	NH$_4$OH	0·147	0 461	}	Brustlein
,,	...	...	NH$_4$OH	0 054	0·386		

In these experiments, the influence of temperature on the equilibrium was only small but the absorption decreased with the rise of temperature, as already shown by Schuhmacher. Equilibrium was very quickly attained. Wiegner concludes that the fixation of bases by soils is best explained as an adsorption phenomenon.

The explanation usually put forward is that the substance actually absorbed is the hydroxide. The OH can be replaced by other ions such as the HCO$_3$ but not usually by those of strong acids. The reaction taking place between the soil and the neutral salt solution consists in the establishment of an equilibrium between the various hydroxides and since the acid radical is not absorbed to any appreciable extent some of the hydroxides must be brought into solution.

Lachs and Michaelis[2] have shown that when charcoal is shaken with alkali, much adsorption takes place— the OH ion being strongly absorbed. If neutral salts are now brought into contact with the charcoal no

[1] *Journ. f. Landw.* 1912, **60**, 111.   [2] *Zeits. f. Elektrochem.* 1911, **17**, 1.

adsorption of the anion takes place except in the case of phosphates but the cations are absorbed by exchange. If acid is brought into contact with the charcoal previously treated with alkali the H ion is strongly absorbed and afterwards the charcoal will no longer take up cations from neutral solutions.

These reactions are obviously parallel to those of Van Bemmelen described previously, and to the general type of absorption in soils by exchange of bases.

The reaction between the soil and phosphates has also been found to obey the adsorption equilibrium law--in this case the phosphates must be kept in solution by using another acid such as dilute hydrochloric or nitric acid as a medium for the reaction. The results[1] indicate that equilibrium obtained between soils and dilute acid solvents such as 1 % citric acid is of an adsorption type. In the case of citric acid there are further complications in that citric acid itself is absorbed by the soil and in this way influences the absorption of phosphate.

In the case of nitrates, sulphates, and chlorides, the absorption of the acid radical has not been noted, all the results obtained so far indicate that no absorption by soil takes place. Even in very dilute solution the author has not found any appreciable absorption of nitrate by soil.

100 gms. of the deep subsoil as used for the oxalic acid absorption (p. 123) were treated with 500 c.c. of a solution containing nitrate. Absorption if any was very small.

N added as nitrate	N found in solution as nitrate*
0 gm	·00036 gm.
·010 gm.	·010 ,,
·020 ,,	·020 ,

* Determined colorimetrically

Mention has been made of percolation experiments for the investigation of absorption phenomena. O. Schreiner and G. H. Failyer[2] obtained the leaching curves for monocalcium phosphate which had been added to a soil. Assuming that the rate of leaching, represented by the concentration of the percolate at any moment, is proportional to the amount of absorbed substance still to come out, then

$$\frac{dy}{dv} = K(A - Y),$$

[1] See this *Journal*, p. 65, and *Proc. Chem. Soc.* 1914, **30**, 123.

[2] *Journ. Phys. Chem.* 1906, **10**, 239.

where $A =$ original amount of absorbed substance,

$Y =$ amount already removed,

$K =$ a constant.

Results were obtained in fair agreement with this assumption. From the adsorption isotherm the rate of removal of absorbed substances can also be worked out.

The concentration of the percolate at any moment must be related to the equilibrium existing between the percolate and the soil.

Using the same expressions as above:

$$\frac{dy}{dv} = K (A - Y)^{\frac{1}{p}}$$

is seen to be another form of the adsorption isotherm. This equation still requires experimental confirmation.

Applications to soil problems.

The surface of soils. The absorption of dyes by soils is a purely physical phenomenon and hence depends largely upon the surface of the soil. A number of workers, Sjollema, Endell, König, Hasenbäumer and Hassler, have suggested the use of this method as means of estimating the colloids of the soil, assuming that only the colloids in the soil take part in the absorption, which is hardly justifiable. J. A. Hanley[1] has reviewed this method and obtains equilibrium curves of the usual type. Where such an equilibrium is established it is obviously difficult to choose a suitable concentration of solution and Hanley points out that the final equilibrium concentration of the solution must be the same for every soil in order that the absorptions may be compared. T. Tadokoro[2] has obtained similar results and finds that the absorption of dyestuffs is closely related to the hygroscopicity—but has no regular relationship to the absorption of ammonia by the same soils.

Soil acidity.

The acidity of some soils has also recently been attributed to absorption phenomena- -in the case of humus soils; on the one hand we have the view that the acidity is due to free acids, on the other hand that it is due to a physical absorption of bases by the humus itself leaving the acid free. Many soils give an acid reaction when brought into contact with moistened litmus paper- the suggestion has been made by Harris[3] and others[4] that this is due to the absorption

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[1] This *Journal*, 1914, **6**, 58.   [2] *Journ. Tohoku Imp. Univ. Sapporo, Japan*, 1914, **6**, 27.
[3] *Journ. Phys. Chem.* 1914, **18**, 355.   [4] See F K. Cameron, *The Soil Solution*, p. 61.

of the base of the litmus by the soil while the free acid is left on the paper. Many soils which give neutral solutions to water give acid extracts to neutral salt solutions and Veitch[1] shows that in some cases of sour soils, iron and alumina are present in the extract. Salts of these bases are easily hydrolysed, giving acid solutions; the acidity of the soil therefore is indicated by the circumstances that the iron and aluminium occur in a form easily turned out by another base. G. Daikuhara[2] has shown that in many cases the aluminium present is equivalent to the acidity. The acidity of the extract also varies with the concentration of the neutral salt solution used, there being a normal absorption equilibrium.

With most English soils showing acidity or sourness the defect can usually be remedied by the addition of lime or chalk to the soil; H. B. Hutchinson and K. MacLennan[3] have shown that such soils have a high absorptive power for calcium bicarbonate solution and they use the expression "lime requirement" to indicate the amount of bicarbonate necessary to saturate the soil.

It will be seen that there is a general tendency to associate all soil absorptions with the general phenomenon of adsorption, and indeed all the characteristics of adsorption are reproduced in most soil absorptions. Whitney and Cameron lay great emphasis on adsorption as the factor determining the composition of the soil solution although it may be doubted whether absorption suffices to keep the concentration of the soil solution within certain limits.

The actual portions of the soil which take part in the various types of absorption have yet to be determined—probably all of them play a certain part - even sand shows a measurable adsorption for dyestuffs. The absorption of bases by exchange—the *Basenaustausch* of the Germans—is more complicated, but the phenomenon is shown by most diverse absorbents. In the soil there probably exists a complex of silica, alumina, possibly humus and iron oxide, which behaves like Van Bemmelen's gels or Way's complex silicates. Certainly combinations exist in soils which are easily decomposed in the cold by dilute acids with the appearance of aluminium and silica in the solution.

In another paper the influence of absorption on analytical methods is demonstrated and probably we are working towards a more precise definition of available plant foods based on our knowledge of how the soil absorbs them in the first instance.

[1] *J. Amer. Chem Soc* 1904, **26**, 637.

[2] *Bull. Imp. Cent Ag. Expt. Stat Japan*, **2**, 1   [3] *This Journal*, 1915, **7**, 75.

THE FRUITING OF TREES IN CONSECUTIVE SEASONS.

BY SPENCER PICKERING, M.A., F.R.S.

(Woburn Experimental Fruit Farm.)

POINTS of considerable interest, both scientific and practical, are raised by the question as to whether a tree which fruits exceptionally well as compared with its fellows in one season, will tend to fruit exceptionally well, or the reverse, in the following season. We know of no definite reason why the behaviour of a tree as regards fruiting should alternate in consecutive years, and no such behaviour has been observed in the case of animals. Its doing so would imply that fruiting is due to the gradual accumulation of some substance in the tree, which becomes exhausted whenever heavy bearing occurs, and that the stock of this substance does not become properly replenished till after another season has elapsed. This is quite possible, but quite unproven. Indeed it seems to be actually opposed to the well established fact that growth and fruiting are antagonistic to each other, for the exceptionally feeble growth which accompanies exceptionally heavy cropping, must tend, as all restriction of growth does, to the formation of an increased number of blossom-buds for the following season, and probably, therefore, to heavy cropping also, during that following season; unless of course, the cropping has been so heavy as to seriously impair the vigour of the tree; but this is an excessive condition which need not be considered here. We might also expect similar behaviour as regards fruiting in consecutive years, on the general grounds that individual trees must differ from each other in fertility, as in every other respect. On the other hand, there is a strong belief amongst horticulturists that a tendency to alternate fruiting, as it may conveniently be termed, does really exist; so much so, that the recommendation is often made, to severely thin the fruit from a tree which is bearing heavily, with the object of destroying this tendency, and of obtaining moderate and more uniform bearing in future years.

One observation which lends some support to such a view is that the prevention of a young tree from bearing for a year or two after

it is capable of doing so, results in its subsequently bearing very heavy crops. From some apple-trees of a precocious variety which had been disblossomed for two years longer than their fellows, the crops obtained during the succeeding five years were, on the average, 3·3 times as heavy as those from their neighbours; after which time their superiority disappeared (*Fifteenth Report*, Woburn Experimental Fruit Farm, 1916, p. 2). It may be argued, however, that the delaying of the first coming into bearing of a young tree is not altogether similar to the restriction of the crop of a tree which is already in bearing.

The difficulties in obtaining exact measurements of the relative behaviour of any two or more trees in consecutive seasons are considerable. The weight of fruit or the number of fruits may be recorded, but, unless the trees are absolutely similar in size, disposition of branches, situation, etc., which is impossible, the superiority of the one over the other as regards inherent fruiting capabilities may be misleading. On the other hand, the trees under observation may be classified by inspection, grouping them according to the extent which they are loaded with fruit, independent of their size, and then comparing the classification in one year with that in the following year. This method avoids errors due to differences in the size of the trees, but it has the disadvantage of being based on the judgment of the observer, and not on actual weights. This latter method was adopted in the case of one series of observations on trees at Harpenden, and the former in the case of the other series on trees at Richmond.

Whichever method of observation was adopted the results were treated as follows: two selected trees were numbered 1 or 2, according to which of them bore the heavier crop in consecutive seasons; then, comparing the results in two consecutive seasons, if the order of fruiting had been the same (consecutive fruiting), the difference between the numbers given to the trees would be 0, if the order had been reversed (alternate fruiting) the difference would be 1; whereas if neither consecutive nor alternate fruiting prevailed, and the results were dependent solely on chance or on external conditions, the differences would, on the average, be 0·5. An average difference, for instance, of 0·75 would be half way between 1 and 0·5, indicating that the results were dependent on the alternating tendency to the extent of 50 per cent., and on chance fruiting to the extent of 50 per cent.

All the trees compared together were of the same age and had received exactly the same treatment since they had been planted; in some cases they were compared in couples, in other cases, the groups

compared together consisted of three or six trees; in which latter cases the actual differences in the "numbers" given to the trees would not be the same as where two trees were compared; but the percentage tendency towards the different forms of fruiting need alone be given here. These are entered in the following table, the first series applying to dwarf apples and pears (in smaller numbers) of several different varieties at Harpenden, the others to dwarf apples at Ridgmont. The dates to which the observations apply are entered in the second column, and the number of instances on which the results depend, in the third column

	Varieties	Dates	Instances	Fruiting indicated		
				Consecutive	Alternate	Chance
1.	Several	1899–1903	332	0	44	56
2	Bramley	1904–1913	1050	12	0	88
3	Cox	1898–1909	840	16	0	84
4	Potts	1897–1904	1266	5	0	95
5	Stirling	1897–1913	366	0	6	94
6	117 varieties	1906–1913	1207	15	0	85

There can be no doubt but that the results at Harpenden are very different from those at Ridgmont, for, whereas the former indicate a strong tendency towards alternate fruiting, the latter, with one exception, indicate a tendency, though a feeble one, towards consecutive fruiting. The Harpenden results must however be discounted to a certain extent, for the number of instances available are smaller, and the period over which the observations extend is shorter than in any of the other cases: also, there were some preliminary observations made on these same trees in 1894–1897, when the results were of an opposite character, there being 20 instances supporting alternate cropping, and 30 supporting consecutive cropping. This difference, however, was probably due to the youth of the trees at the time, for, with trees which have not yet come into proper bearing, precociousness, or the reverse, in certain individuals, would increase the number of trees behaving in the same way in consecutive seasons.

The difference between the Harpenden and Ridgmont results can not, however, be explained away on any of the above grounds, and are, no doubt, real. There are probably tendencies both to alternate and consecutive cropping, and whether the one or the other predominates, depends on the conditions of soil, climate, etc. There can be no doubt, however, that the main factor determining the fruiting of trees in consecutive years is neither of these tendencies, but chance, that is, external conditions: with the Ridgmont results, this is potent to the

extent of about 90 per cent., and, even with the Harpenden results, it is the predominant factor.   That it should be more potent at Ridgmont than at Harpenden is easily explained, for the former of these stations is much more subject to spring frosts than the latter, and it is to spring frosts that the failure of crops is generally attributable.

In the case of the plantation of 117 varieties of apples there were eight trees of each variety, and these were divided into pairs, one pair on the paradise stock, which had been very lightly pruned since they were planted, and one pair on the same stock, which had been more severely pruned; there were also two pairs on the crab stock, which had been treated in like manner.   An examination of the results indicates that the tendency towards consecutive bearing becomes more marked as the age of the trees increases: thus, dividing the results into two periods, 1906–1909 and 1909–1913, it was found that the tendency in this direction, taking all the trees together, amounted to 8 per cent. for the first period, and 26 per cent. for the second, and the differences were uniformly exhibited by each of the four classes of trees: this is what might be expected, for any habit exhibited by a plant or animal tends to become more established as its age increases.   As to the influence of stock; trees on the paradise stock uniformly showed a smaller tendency towards consecutive fruiting (11 per cent.) than did those on the crab stock (30 per cent.); whilst as regards pruning, the results were equally uniform, the tendency towards consecutive fruiting being less marked with the more severely pruned trees (10 per cent.) than with those very lightly pruned (26 per cent.).   Apparently, the more a tree is left to natural development, the more does its fruiting become conditioned by extraneous circumstances.

In the case of observations on a plantation containing different varieties of trees, it is probable that the influence of chance on the bearing of the trees will be unduly exaggerated; for some of the instances which counterbalance each other will consist of cases where the consecutive and alternate fruiting is attributable to the inherent properties of the trees, and not merely to the effect of chance or extraneous circumstances.   From the results of general, though, perhaps, not very exact, observations made by fruit growers, it appears fairly certain that some varieties of apples do exhibit a marked tendency towards alternate fruiting, and, doubtless, there are other varieties which exhibit an equally marked tendency towards consecutive fruiting, though such instances, naturally, do not attract special attention.   It would be necessary, therefore, in a strict examination of this subject, to treat

the results with each variety separately. This has been possible in the case of four sets only (2 to 5 in the above table), and, consequently, more weight must be attached to these than to the results from those of the mixed plantations, 1 and 6.

The good and poor fruiting of a plantation as a whole in alternate seasons, must argue against any tendency in the individual trees towards alternate fruiting, for it shows that this alternation of bearing has been determined by some extraneous circumstance which has affected all the trees alike; whereas if there were any innate tendency towards alternation, it would not be exhibited by different individuals in the same year: in a plantation consisting of many individuals, therefore, even of the same variety, an average uniformity of production would result. As already stated, the chief factor affecting the cropping at Ridgmont is the incidence of spring frosts, and there certainly appears to be a tendency for these frosts to recur in alternate seasons, causing an alternation in the cropping of the plantations. The results with Stirling Castle, Bramley and the Variety plantation are set out below, the numbers giving the relative magnitude of the crops compared, in the case of Stirling Castle, with the crop in 1900 as 100, and, in the other cases, with that of 1911 as 100.

	1897	1898	1899	1900	1901	1902	1903	1904	1905	1906
Stirling......	3	30 +	6 −	100 +	47 −	144 +	0 −	148 +	0 −	144 +
Bramley ...								35	6 −	7 −
Varieties ...								0	0 −	12 +

	1907	1908	1909	1910	1911	1912	1913	1914	1915
Stirling......	38 −	95 −	171 −	264 +	78 −	47 −	207 +	0 −	549 +
Bramley ...	20 +	7 −	103 +	9 −	100 +	0 −	103 +	0 −	159 +
Varieties ...	20 +	15 −	34 +	13 −	100 +	10 −	114 +	0 −	114 +

A plus or minus sign has been placed after the values showing whether they are above or below the means of the preceding and succeeding seasons, and the extent to which these signs alternate, though not without some irregularities, is very remarkable; and this alternation may now be extended by another season, for the crops this year are far below the average. In the case of Cox and Potts no such alternations were recognisable, but, of course, owing to differences in the dates of blossoming, and the hardiness of the flower buds, all varieties would not be affected to a like extent by frost in any given season.

NOTE ON THE LOSS OF PHOSPHORIC ACID DURING FUSION WITH AMMONIUM FLUORIDE.

By WILLIAM A. DAVIS and JAMES ARTHUR PRESCOTT.

(*Rothamsted Experimental Station.*)

In the analysis of soils and rocks, treatment with hydrofluoric acid or ammonium fluoride is frequently prescribed for the estimation of constituents other than silica (compare for example Wiley's *Agricultural Analysis*, Vol. I, p. 352). Recent results obtained by the writers show that this method fails to give reliable results in the case of phosphoric acid owing to a loss of this constituent during the volatilisation of the silica as silicon tetrafluoride. The exact cause of the loss was not investigated, but apparently in presence of an excess of ammonium fluoride or hydrofluoric acid some of the phosphorus may volatilise in the form of phosphorus trifluoride or phosphorus pentafluoride. The loss appears to be least with phosphates of the alkali metals, the highly basic nature of the metal enabling it better to retain the phosphate radicle. The loss from phosphates of the alkaline earths (lime) during treatment with the fluoride is considerably greater and it is very marked, and may rise to over 50 % in the case of minerals such as apatite.

This loss was first observed during the determination of phosphoric acid in a sample of pottery waste. The ordinary hydrochloric acid digestion process showed this material to contain 11·54 % of total P_2O_5. When 1 grm. of the sample was fused in the usual way with 8 grms. of pure ammonium fluoride so as to volatilise the whole of the silica, the residue being gently ignited twice with sulphuric acid, then dissolved in dilute hydrochloric acid or nitric acid, and made up to a known volume (200 c.c.), very variable and low results were obtained. Thus in three successive experiments the sample appeared to contain only 7·37, 7·96 and 6·85 % of P_2O_5.

Special experiments show that this loss of phosphoric acid does not occur during ignition with sulphuric acid as carried out in the later part of the experiment. It was only obtained when there had been previous treatment with ammonium fluoride.

Experiments with Sodium hydrogen phosphate, Na_2HPO_4, $12H_2O$.

The specimen of sodium hydrogen phosphate used had been carefully recrystallised by Prescott[1] in his work on the estimation of phosphoric acid by the molybdate method. It had, however, slightly effloresced so that when 1 grm. was dissolved in 200 c.c. of water 100 c.c. of the solution contained 0·1096 grm. P_2O_5 instead of the 0·0990 grm. corresponding to the formula Na_2HPO_4, $12H_2O$.

Several experiments were made with the salt, in each case 1·000 grm. being fused with 8 grms. of pure ammonium fluoride; the residue then remaining was then gently ignited twice with sulphuric acid to expel all the hydrogen fluoride, and dissolved in dilute nitric acid, and made up to 200 c.c. The results obtained were variable, showing varying losses of phosphoric acid. Thus, in the successive experiments, 100 c.c. of the final solution contained 0·0989, 0·0886 and 0·0942 grm. P_2O_5, *i.e.* 90·2, 80·9 and 86·0 % of the phosphoric acid originally present in the salt.

Experiments with Potassium dihydrogen phosphate, KH_2PO_4.

With this salt, containing a smaller proportion of the alkali metal, the loss of phosphoric acid during the treatment with ammonium fluoride was considerably greater than with the disodium salt.

0·500 grm. of the commercially pure (Kahlbaum's) salt dissolved in 100 c.c. of water gave a solution containing 0·2539 grm. P_2O_5, as estimated by the molybdate method.

When 0·5000 grm. of the salt was fused with 4 grms. of pure ammonium fluoride and treated as in the case of the sodium phosphate, being finally made up to 100 c.c., quantities of the P_2O_5 varying between 0·1623 and 0·2078 grm. were found in the 100 c.c. Thus only 63·9 to 81·9 % of the phosphoric acid remained after the treatment with fluoride.

Experiments with Calcium phosphate.

Kahlbaum's precipitated calcium phosphate was used. 0·5000 grm. dissolved in 200 c.c. of dilute nitric acid gave a solution containing 0·2030 grm. P_2O_5, as estimated by the molybdate method.

0·500 grm. of the same material was fused with 4 grms. of ammonium fluoride, and after volatilising the excess of the latter, ignited twice with sulphuric acid and the residue dissolved in dilute

[1] This *Journal.* 1914, **6**, 111

nitric acid; the solution after being diluted to 200 c.c. was found to contain only 0·0983 grm. of P_2O_5 in the 200 c.c., that is 42·5 % of the weight of the phosphoric acid originally taken.

The following experiment showed that the loss did not occur when the fusion with ammonium fluoride was omitted. 0·500 grm. of the same material was ignited twice with sulphuric acid and the residue dissolved in dilute nitric acid; the solution after dilution was found to contain 0·201 grm. P_2O_5; that is 99·0 % of the weight of phosphoric acid originally taken.

Experiments with Apatite.

0·500 grm. of the apatite was dissolved in dilute nitric acid and the solution made up to 200 c.c.; the total P_2O_5 in 200 c.c. was found to be 0·2062 grm.

When 0·500 grm. of the same sample was fused with 4 grms. of ammonium fluoride and treated as in the case of the calcium phosphate, the 200 c.c. of solution finally obtained contained only 0·0928 grm. P_2O_5 or 45·0 % of the quantity originally taken.

SUMMARY.

1. When salts or mineials containing phosphoric acid are ignited with ammonium fluoride as in the ordinary process of analysis of silicates, considerable loss of the phosphoric acid may occur. It is probable that the phosphorus is volatilised in the form of a phosphoius fluoride.

2. The loss is least in the case of salts containing an alkali metal. It is less in the case of disodium hydrogen phosphate than in that of potassium dihydrogen phosphate, and is greatest in the case of phosphates of the alkali earth metals, such as calcium phosphate or apatite.

STUDIES IN MILK SECRETION.

BY J. HAMMOND, M.A., AND J. C. HAWK, B.A.

(School of Agriculture, Cambridge.)

I   THE EFFECT OF NUTRITION ON YIELD AND COMPOSITION.

INTRODUCTION.

MUCH work has been done on the effects of various foods and rations on milk secretion both as regards yield and composition. It has, however, with one or two exceptions, failed to establish clearly any fundamental principles with regard to the effect of nourishment on milk production.

For the most part the experimenters have been working with cows kept partially for commercial purposes and so they have been unable to go to the extremes necessary for the elucidation of fundamental principles.

Ingle[1] found that foods rich in albuminoids improved both the yield of milk and proportions of fat and solids not fat, while large quantities of carbohydrates although increasing the yield appeared to diminish its quality. Crowther[2] demonstrated the fact that a change from a highly nitrogenous diet to one relatively poor in nitrogen resulted in the secretion of more milk but that this was poorer in fat content; the reverse of this—a change of diet to one relatively rich in nitrogen—was followed by a decrease in the yield but an increase in the fat content.

Physiologists, while studying the origin of the lactose of milk, have found that as a result of limiting the supply of sugar in the blood, by the action of drugs, the yield of milk has been affected.

Lusk[3] found that phloridzin when administered to a goat caused a decrease in the yield of milk but the percentage of fat was increased.

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[1] *Bul. Yorks. Coll.*, Leeds, No. 25, 1901.   [2] *Univ. of Leeds, Bul.* No. 44, 1903.
[3] *Ztschr. f. Biol.* Bd. XLII, 1901.

Porcher[1] found that on giving phloridzin to lactating goats and cows the yield of milk for the following periods was decreased in amount. Paton and Cathcart[2] by the same means decreased the amount of glucose available in the blood of lactating goats and found that this resulted, not in a decrease of the percentage of lactose in the milk, but in a diminished secretion of milk together with amounts of lactose, nitrogen and ash formed. They found that the lowest secretion of milk took place on the day that the largest output of sugar occurred in the urine.

Rose[3] found that the volume of milk secreted fluctuated inversely with the amount of phytin in the food and that the composition was not altered except that the percentage of fat increased when the phytin was administered.

METHODS.

The object of our investigation was to study the changes in the yield and composition of the milk which followed a sudden change in nutrition. The sudden changes in nutrition were brought about by the administration of phloridzin together with the control of the food supply.

Well fed goats were used in all the experiments and care was taken that they had continual access to an abundant supply of water. They were milked regularly at different intervals three times a day; records were kept of the yield at each milking in c.c. and the percentage fat in each milking was estimated by the Gerber method. The daily figures only are given in the tables below; the percentage fat for each day being calculated from the total fat and daily yield.

EXPERIMENTS.

Three series of experiments were performed: (a) food was withheld for a short time and then a plentiful supply given; (b) food was withheld, phloridzin was injected and shortly afterwards a plentiful supply of food given; (c) phloridzin was injected into goats under perfectly normal conditions of feeding.

Series (a). Food was withheld from well nourished goats for a few days and then an abundant supply given them. Two experiments were undertaken and Table I below shows the results obtained.

[1] *Arch. Internat. de Physiol.* T. VIII, 1909.
[2] *Jour. of Physiol.* Vol. XLII, 1911.
[3] *New York State, Sta. Tech. Bul.*, No. 20, 1912.

TABLE I.

Exp. I.   Goat III.

Days ...	1	2	3	4	5	6	7	8	9
Remarks ...	—	—	Food taken away	—	—	—	—	Food given	—
Yield in c.c. ...	91	86	107	56	45	54	45	61	101
% fat ...	2·85	3·41	3·43	5·05	5·40	6 30	8·69	7·01	3·57
Amt. fat, gm. ...	2·60	2·97	3·67	2·83	2·43	3·4	3·91	4·28	3·61

Exp. II.   Goat II.

Remarks ...	—	Food taken away	—	—	Food given	—	—	—	—
Yield in c.c. ...	196	98	59	50	36	105	128	146	184
% fat ...	5·37	10·21	11·96	13·26	11·0	6·22	5·39	5·72	5·41
Amt. fat, gm. ...	10·53	10·01	7·06	6·63	3·96	6·53	6·90	8·36	9·96

It will be seen that the result of lowering the state of nutrition was that the *amount* of milk and fat secreted *decreased* whereas the *percentage* of fat *increased*.

Series (b). Food was withheld from well nourished goats for a short time and then injections of phloridzin (in alcohol) made; a short time after an abundant supply of food was given. Three experiments were undertaken and the results of these are given in Table II below.

TABLE II.

Exp. I.   Goat II.

Days ...	1	2	3	4	5	6	7	8	9	10	11
Remarks ...	—	Food taken away	1 gr. phloridzin	—	Food given	—	—	—	—	—	—
Yield in c.c. ...	231	136	65	3	27	64	82	87	110	160	—
% fat ...	7·65	13·28	17·14	19·67	19·22	11·50	5·62	7·94	5·24	4·86	—
Amt. fat, gm. ...	17·69	18·07	11·14	59	5·19	7·39	4·61	6·91	6·23	7·78	—

Exp. II.   Goat III.

Remarks ...	—	Food taken away	—	4 grs. phloridzin	—	Food given	Abortion	—	—	—	—
Yield in c.c. ...	127	73	61	12	3	0·4	7	18	24	35	48
% fat ...	4·37	5·67	5·73	16 91	21·0	?	11·86	13·05	9·04	7·34	5·08
Amt. fat, gm. ...	6·19	4·14	3·5	2 03	·63	?	·83	2·35	2·26	2·67	2·44

Exp. III.   Goat I.

Remarks ...	—	Food taken away	1 gr. phloridzin	Food given	—	—	—	—	—	—	•
Yield in c.c. ...	225	117	27	35	129	83	107	80	—	—	—
% fat ...	11·51	13·69	17·96	20 97	9·87	8·85	7·27	6·36	—	—	—
Amt. fat, gm. ...	25·9	16·01	4·85	7·34	12·74	7·35	7·78	5·09	—	—	—

The results are similar to those of the last series of experiments but the action is more marked. It will be seen that the yield of milk in no case has returned to normal within the few days of the experiment though it did eventually. It would seem that phloridzin has a rather long-continued action; this confirms the work of Paton and Cathcart who found sugar in the urine for a long time after the administration of phloridzin.

In two cases out of the three the fat percentage at the end of the experiment was below that at the beginning, although the daily yield of milk had not returned to normal.

Series (c). Phloridzin was injected into goats under normal conditions of feeding. It was thought that this would reduce the amount of stored carbohydrates in the body and perhaps thereby limit the amount of milk secreted. Table III below gives the results obtained in the four experiments performed.

TABLE III.

Exp. I. Goat II.

Days	1	2	3	4	5	6	7	8	9
Remarks	—	2 grs. phloridzin	2 grs. phloridzin	—	—	—	—	—	—
Yield in c.c.	200	228	260	252	124	—	—	—	—
% fat	7·84	7·40	7·70	8·93	12·25	—	—	—	—
Amt. fat, gm.	15·68	16 88	20·02	22·52	15·19	—	—	—	—

Exp. II. Goat I.

Remarks	—	1 gr phloridzin	—	—	—	—	—	—	—
Yield in c.c.	81	24	29	55	56	59	62	64	87
% fat	7·47	11·95	9·52	7·27	6·71	6·42	6·24	5·57	5·48
Amt. fat, gm.	6 05	2·87	2·76	4·38	3·76	3·79	3·87	3·57	4·77

Exp. III. Goat IV.

Remarks	—	—	2 grs. phloridzin	—	—	—	—	—	—
Yield in c.c.	960	933	940	837	860	944	—	—	—
% fat	3·90	3·58	3·93	3·62	3·60	3·93	—	—	—
Amt. fat, gm.	37·44	33·39	37·03	30·34	31·00	37·07	—	—	—

Exp. IV. Goat V.

Remarks	—	—	2 grs. phloridzin	—	—	—	—	—	—
Yield in c.c.	617	557	536	324	465	526	—	—	—
% fat	3·98	3·84	4·09	5·29	3·67	4·10	—	—	—
Amt. fat, gm.	24·58	21·42	22·08	17·17	17·10	21·57	—	—	—

It will be seen that there are great variations in the effect on different individuals, and observation pointed to the conclusion that it was the goats in the worst states of nutrition that showed a diminution in the milk yield as a result of injection. Two explanations of this appear to be possible, either (1) in the goats in a high state of nutrition the loss of sugar by way of the kidney was not sufficient materially to alter the amount of sugar in the blood and so the amount available for the mammary gland, as would occur in goats with very little stored carbohydrate; or (2) the milk yield was only affected when the proteins were disorganized to yield sugar and that the store of carbohydrates in the well nourished goats acted as a shield to delay this disintegration.

In either case it is evident that interference with the sugar metabolism affected not only the amount of milk sugar but also the amount of milk and protein without affecting to any great extent the amount of fat produced.

DISCUSSION.

The results of the experiments obtained in Series (b) have been collated by interpolation of days and the results averaged and plotted in the form of a curve. The curves (Fig. 1) show very clearly the effect of lowering the state of nutrition on the yield and fat content.

These results have a direct interest in view of the frequency with which dairymen are prosecuted for selling milk deficient in fat. The defence is often put forward that the milk was poor in fat in consequence of a dry season and the shortage of feed. The results of our experiments show on the contrary that, under such conditions of a reduced state of nutrition, the milk is very rich in fat. It will be seen above however that if, after such a period of low feeding, an abundant supply of food is given the yield is increased but the percentage of fat is below normal. This conclusion is in agreement with that of Eckles[1], who found that provided a cow is underfed after calving the percentage of fat in the milk is influenced to a considerable extent by the fatness of the cow at calving. Surveying the feeding experiments on dairy cows from this point of view one sees repeated examples of this principle demonstrated. Wakerley[2] found that, although the yield of milk was greate as a result of feeding Golden Tankard mangolds to cows, the quality of it was impaired, but the total amount of butter fat was the same

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[1] *Univ. of Missouri, Agric. Exp. Sta. Bul.*, 100.

[2] *Rpt. Midland Agric. and Dairy Coll.*, 1905–6.

as that of the control lot. Lucas[1] found that cacao husk when fed to milch cows diminished the milk yield 20 per cent. but it increased the fat content 20 per cent. per volume; this showing that the amount of fat was not affected but that the cacao husk was deficient in nourishment suitable for milk formation.
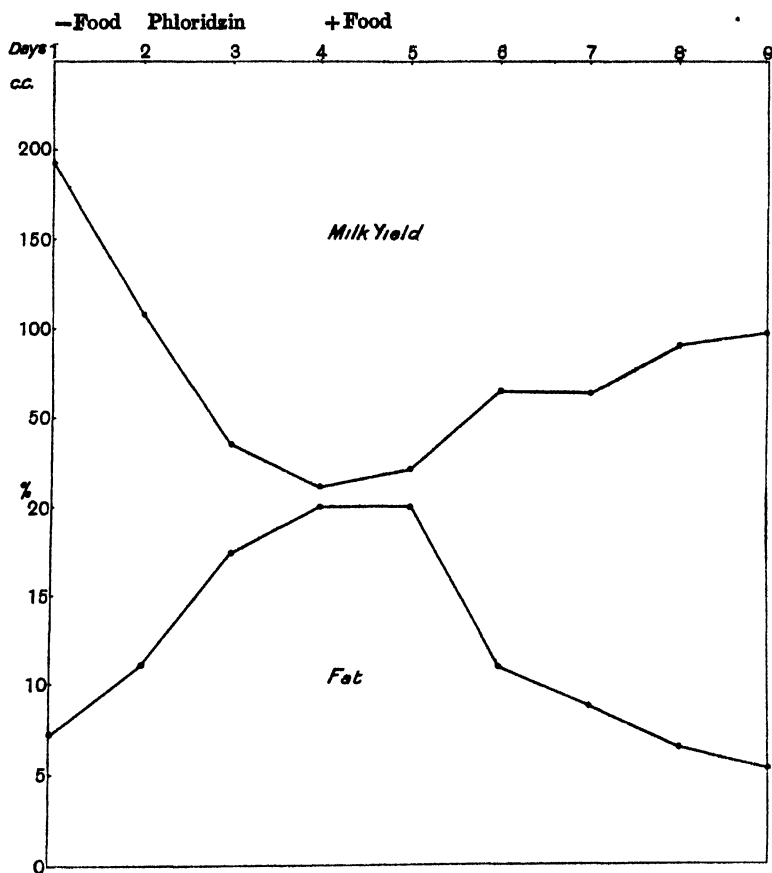
Fig. 1.

It is interesting to compare the foregoing changes in the yield and composition of milk brought about by nutrition with those occurring during the period of lactation. It has recently been shown by several investigators that the growth and development of the mammary glands is more especially under the control of the corpus luteum. It is probably the influence of this body on the nutrition of the mammary

[1] *Ann. d. l. Sci Agron.* 4e Séne, T. i, p. 321, 1912.

gland which causes the changes in yield and composition assigned to the period of lactation. The curves showing differences in yield and composition due to the period of lactation in Fig. 2 below are taken from a paper by Crowther and Ruston[1] and should be compared with the curves given in Fig. 1. A comparison of these two curves shows that the changes in yield and composition due to "nutrition" and "lactation" are essentially similar, except in the initial period due possibly to the "condition" of the cows at calving. Possibly those changes due to "lactation" are really due to nutrition controlled
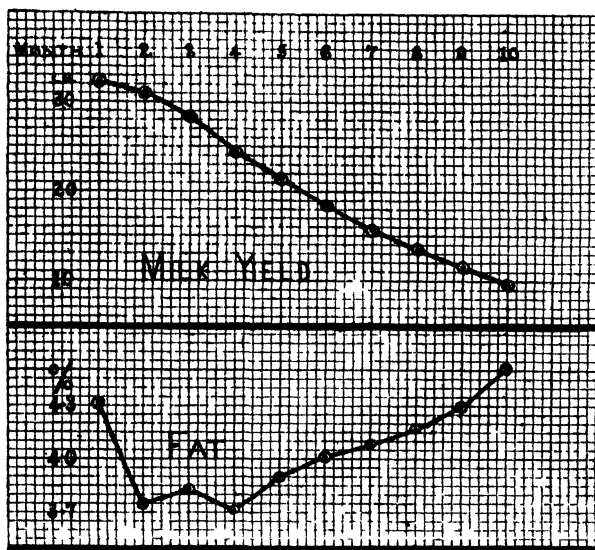
Fig 2.

internally by the corpus luteum. If such is the case one would expect the food supply to play a much more important part in influencing the yield during the height of the lactation period, since the other limiting factor ("internal nutrition") will be at its maximum   As an example of this in actual practice Bryner-Jones[2] found that a ration containing brewer's grains had a relatively greater effect on the milk yield of cows earlier in the lactation period than it did later on.

[1] *Trans High and Agric Soc of Scotland*, Vol. xxiii, 1911.
[2] *Durham C.C , Offerton Bul* No 2, 1907

SUMMARY AND CONCLUSIONS.

I. As a result of withholding food for a few days, together with an injection of phloridzin, thereby reducing the nutrition, the daily yield of milk in goats was diminished and in one case the flow was actually stopped. On giving food again the yield returned almost to normal within a few days.

II. As the daily yield of milk diminished under these conditions so the percentage of fat in the milk rose. Limitation of the available nutriment in the body (change from a high to low state of nutrition) did not reduce the percentage of lactose or protein in the milk (Paton and Cathcart[1]) but reduced the quantity of milk (together with the amounts of protein, sugar and salts) produced. The secretion of fat was not at first affected by the change in metabolism and as a consequence milk rich in fat was produced.

III. The amount of fat secreted per day under these conditions of diminishing yield was however not constant but became reduced, possibly as a secondary effect of the decreased secretion taking place in the gland cells.

IV. On again giving food to animals in such a reduced state of nutrition, the percentage of fat in the milk decreased as the yield increased, in some cases to such an extent that it was below that of the normal milk before the experiment began.

[1] *Jour. of Physiol.* Vol. XLII, 1911.

STUDIES IN MILK SECRETION.

By J. HAMMOND, M.A., and J. C. HAWK, B.A.

(School of Agriculture, Cambridge.)

II. THE RELATION OF THE GLANDS OF INTERNAL SECRETION TO MILK PRODUCTION.

INTRODUCTION.

IT is now generally recognized that the glands of internal secretion play an important part in regulating metabolism and so controlling the nutrition of the animal. Hatui[1] found that castration was followed by a putting on of fat except in those cases in which the pituitary underwent a compensatory hypertrophy, when the animal remained normal in weight.

It seems possible that what is known as "individuality" in the fatness or thinness of any animal or breed of animals is an expression of the equilibrium between the various glands of internal secretion in the body. This may also apply to "individuality" in the yield of milk of cows. Injection of extracts of various glands of internal secretion has in some cases a marked effect on milk secretion. Ott and Scott[2] have drawn up the following table, classifying the glands according to the mode of action of their extracts on milk secretion.

Exciting	Inhibitory	Synergistic
Pituitary extract	Adrenalin	Orchitic extract
Corpus luteum extract	Iodothyrin	—
Pineal gland extract	Ovary minus corpus luteum extract	—
Thymus extract	Spleen extract	—
Mammary gland extract	Pancreas extract	—

Experiments have been performed with pituitary extract and with adrenalin, and some of the results are described below. No attempt

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[1] *Jour. Exp. Zool.* Vol. xv, 1913. [2] *Therapeutic Gazette*, p 761. Nov. 1912.

has been made to confirm the conclusions reached by Ott and Scott with regard to the other glands.

PITUITARY EXTRACT.

Effect of nutrition on the flow of milk following an injection.

In a former paper by one of us[1] the action of pituitary extract on the mammary gland was investigated and it was noticed that the effect of pituitary extract varied with the state of nutrition of the animal. Ott and Scott[2] noticed that when a feverish condition arose in a goat injections of pituitary extract acted very feebly. The effect of pituitary extract was therefore studied in animals under conditions of reduced nutrition, as described in the previous paper[3], in the hope that this would throw light on the mode of action of the extract.

Pituitary extract[4], in each case 1 c.c., was injected daily into goats during a period of sudden change from a high to low condition of nutrition. This change was brought about as described in the previous paper[3] and three series of experiments have been performed: (*a*) without food for a few days and then return to abundant nourishment; (*b*) without food for a few days together with an injection of phloridzin followed by an abundant supply of food; (*c*) injections of phloridzin under normal conditions of feeding.

The goats were milked dry each morning and immediately after 1 c.c. of pituitary extract was injected It has previously been shown that under normal conditions injections at intervals of one day give no immunizing effect, injections of 1 c.c. at such intervals giving approximately the same amount of milk. Since it has been previously shown that the action of the extract was complete under half an hour after an injection, the goats were milked after this period, and the amount obtained is taken as the yield resulting from pituitary injection. The percentage of fat in each sample was determined by Gerber's method.

The following tables Nos. I, II and III give the results obtained in the various experiments of the series (*a*), (*b*) and (*c*) respectively:

[1] *Jour. Exp. Physiol.* Vol. VI, 1913.

[2] *Therapeutic Gazette*, October, 1911.

[3] "Studies in Milk Secretion," Part I. This *Journal*.

[4] Messrs Burroughs and Wellcome's extract of the posterior lobe was used in all these experiments.

TABLE I.

Exp. I. Goat III.

Days	1	2	3	4	5	6	7	8	9
Remarks	—	—	Minus food	—	—	—	—	Food given	—
Morning yield, c.c.	63	47	68	31	27	32	23	38	69
Pituitary yield, c.c.	16	20	22	15	12	14	13	18	22
Ratio: morning yield (= 100) to pituitary yield	25	42	32	48	44	45	56	48	31
% fat pituitary milk	3·0	3·8	4·1	6·8	7·5	8·4	13·0	9·5	4·6

Exp. II. Goat II.

Remarks	—	Minus food	—	—	Food given	—	—	—	—
Pituitary c.c.	33	23	19	16	12	17	19	21	25
Ratio: morning yield (=100) to pituitary yield	28	47	71	69	64	22	21	20	20
% fat pituitary milk	8·9	12·0	15·2	15·4	14·4	11·9	9·3	8·9	8·5

TABLE II.

Exp. I. Goat II.

Days	1	2	3	4	5	6	7	8	9	10	11
Remarks	—	Minus Food	Phloridzin	—	Food given	—	—	—	—	—	—
Pituitary c.c.	32	34	21	·75	12	23	20	19	25	30	—
Ratio: morning yield (= 100) to pituitary yield	19	48	84	150	91	64	34·4	35	30·1	27	—
% fat pituitary milk	9·1	15·4	24·0	29·9	18·6	13·3	11·2	9·2	8·6	8·2	—

Exp. II. Goat III.

Remarks	—	Minus food	—	Phloridzin	—	Food given	—	—	—	—	—
Pituitary c.c.	25	18	13	4	·25	drop	2	8	10	10	16
Ratio: morn. yield (= 100) to pituitary yield	29	40	43	133	?	?	50	86	102	53	50·5
% fat pituitary milk	8·2	7·8	9·6	21·7	25·1	?	20·0	14·4	10·1	8·7	7·3

Exp. III. Goat I.

Remarks	—	Minus food	Phloridzin	Food given	—	—	—	—	—	—	—
Pituitary c.c.	63	35	2	16	36	23	22	9	—	—	—
Ratio: morn. yield (= 100) to pituitary yield	49	57	32	127	46	49	32	17	—	—	—
% fat pituitary milk	14·8	18·7	29·8	18·8	12·4	11·5	11·1	10·8	—	—	—

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TABLE III.

Exp. I. Goat II.

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Days	1	2	3	4	5	6	7	8	9
Remarks ...	—	Phloridzin	Phloridzin	—	—	—	—	—	—
Pituitary milk c.c.	40	33	29	40	40	—	—	—	—
Ratio: morn. yield (= 100) to pitui- tary yield	31	21	15	28	57	—	—	—	—
% fat pituitary milk	9·7	8·4	9·7	14·5	14·5	—	—	—	—

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Exp. II. Goat I.

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Remarks ...	—	Phloridzin	—	—	—	—	—	—	—
Pituitary c.c.	18	5	10	13	11	6	11	12	15
Ratio: morn. yield (= 100) to pitui- tary yield	35	54	56	41	26	25	24	24	24
% fat pituitary milk	11·3	16·6	10·1	12·0	7 1	7·6	8·1	8·0	9·8

From the results shown in the previous tables it would seem that the amount of milk produced by the action of pituitary extract varies with the state of nutrition. However the ratio, morning yield to pituitary yield, rises with the fall in nutrition and falls as the nutrition rises again so that the yield obtained as a result of pituitary injections tends to be more constant than the morning or daily yield. Pituitary extract seems to act on some store which is not affected so much by external conditions as is the daily yield. At first sight it would appear possible that this store is situated in the ducts and alveoli of the mammary gland; but if this is so, it is difficult to explain the small effect of pituitary extract on goats in a low state of nutrition, for during these experiments there has been no time for the duct or alveolar space to change.

It will be seen that the percentage of fat in the pituitary milk rises with the fall in nutrition as it does in the case of normal milk till, in some cases, milk with practically 30 per cent. fat has been obtained.

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ADRENALIN.

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It will be noticed that Ott and Scott classify adrenalin as an inhibitor of milk secretion; this seems surprising since adrenalin is known to have many of the same actions on the body as pituitary extract has, causing contraction of plain muscle, hyperglycaemia and glycosuria.

The effect of injections of adrenalin into lactating goats has been tried in order to find out, if possible, its mode of action.

The goats (three) were milked at definite times twice a day but in addition to this injections of, on the average, 6 c.c. of a $\frac{1}{1000}$ solution of adrenalin chloride were made on alternate days after the morning milking, the goats being milked again at an interval of half an hour. On the "normal" days, which alternated with the days on which adrenalin was given, the treatment was exactly the same except that sterilized water was injected in the place of adrenalin. It was so arranged that the day, for the purpose of computing the yield, started with an injection. Table IV below shows the results obtained with the three goats which were treated in this way.

TABLE IV.

Exp. I. Goat IV.

	Days	1–2	3–4	5–6	7–8	9–10	11–12
Normal	Injection c.c.	18	—	18	30	15	18
	Total c.c.	931	700	805	898	925	923
Adrenalin	Injection c.c.	29	11	40	22	21	17
	Total c.c.	664	739	806	752	791	768

Exp. II. Goat V.

Normal	Injection c.c.	—	47	26	11	5	—
	Total c.c.	578	549	511	511	491	—
Adrenalin	Injection c.c.	18	31	30	30	10	—
	Total c.c.	390	473	415	451	353	—

Exp. III. Goat II.

Normal	Injection c.c	—	—	17	23	17	18
	Total c.c.	469	472	521	537	583	594
Adrenalin	Injection c.c.	14	11	8	9	9	12
	Total c.c.	392	407	436	391	450	489

Table V below gives the averages for all these experiments. The fat was estimated on only eight days out of the twelve over which the experiment extended, so the figures given for the percentage of fat and amount of fat are the averages of these days only.

TABLE V. Averages.

	c.c. milk		% fat		gm. fat	
	Adrenalin	Normal	Adrenalin	Normal	Adrenalin	Normal
Injection	19	20	7·9	7·6	1·5	1·5
Evening	122	172	5·5	5·2	6·7	9·1
Morning	388	452	4·1	3·5	16·3	15·8
Total	527	643	4·6	4·0	24·5	26·4

It will be seen that the effect of adrenalin injections is to reduce the rate of milk secretion for the period following that of injection but it has no marked immediate effect on the mammary gland. It is quite possible that the suprarenals produce in the body at times large quantities of adrenalin. Cannon, Stohl, and Wright[1] have found that glycosuria is produced after great excitement or severe fright and this is probably due to increased secretion of adrenalin. It is a well-known fact that cows give less milk under these circumstances. This fact has hitherto been cited as showing that the nervous system has some action on mammary secretion, although investigators have always failed to demonstrate the existence of nerves controlling mammary secretion.

It is interesting to compare the effects of pituitary extract and adrenalin; both cause contraction of involuntary muscle, hyperglycaemia and glycosuria, but pituitary extract alone has the immediate effect of augmenting milk secretion. The main difference in their action is that adrenalin acts only in connection with the sympathetic nervous system and pituitary extract acts generally.

Although the effect on milk secretion immediately obtainable after injection of pituitary extract is not brought about by adrenalin, the latter substance produces the same after-effects, due perhaps to the loss of sugar by glycosuria. Experiments with phloridzin described in the previous paper show also that anything disturbing the sugar metabolism of the body at once affects milk secretion.

CONCLUSIONS.

I.   The flow of milk produced as a result of an injection of pituitary extract varies with the state of nutrition of the injected animal.

II.   This variation (due to nutrition) is not so great as that produced in the case of the morning or the daily yields, indicating that the action of the pituitary extract is on some more stable (storage) quantity.

III.   The percentage fat of the pituitary milk is increased by the state of lowered nutrition in the same way as that of the normal milk.

IV.   Injections of adrenalin though resembling pituitary extract in causing hyperglycaemia differ from them in having no immediate action on milk secretion.

V.   Injections of adrenalin have a secondary effect on milk secretion causing a decrease in the amount of milk produced for a period of a day following its injection.

[1] *Amer. Jour. of Physiol.* Vol. xxix, 1912.

VI. The percentage of fat in the milk from the period following an injection of adrenalin is above normal, although the actual amount obtained is somewhat below normal.

VII. The rate of the milk flow is very susceptible to changes in the sugar metabolism of the animal.

SOME SOILS OF THE SOUTHERN ISLAND OF NEW ZEALAND WITH SPECIAL REFERENCE TO THEIR LIME REQUIREMENTS.

By LEONARD JOHN WILD, M.A., F.G.S.

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I. THE METHOD EMPLOYED.

A. *Introduction.*

THE work of which the following paper is a partial record, was primarily undertaken with a view to testing the applicability to New Zealand soil conditions of a method described by Hutchinson and MacLennan[1] for determining the lime requirements of soils. The method consists essentially in treating a known weight of soil for three or four hours with a known volume of a solution of bicarbonate of lime of known concentration, and afterwards determining by titration with standard acid the loss of lime suffered by the solution. This loss of lime is deemed to be the lime requirement of the soil. Owing to the scarcity and consequent high cost of labour in New Zealand, and owing to the distance of lime deposits from most of our areas of arable soils, chalking, marling, or liming the soil in any other manner has never been resorted to in this country on such an extensive and wholesale scale as is frequently practised in Great Britain. Of late years, however, the question of the necessity or otherwise of increasing the lime content of our soils, has been keenly debated, and the need of some scientific method of settling the question has become acute. Hence the writer eagerly seized upon the method suggested which, on account of its own inherent reasonableness, and the status of its sponsors, as well as because of its rapidity and ease of manipulation, promised to be of the greatest

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[1] "Studies on the Lime Requirements of Certain Soils," *Journal of Agricultural Science*, Vol. VII, Part I (March, 1915), p. 75.

assistance in elucidating an important problem for agriculturists in New Zealand.

B. *Laboratory Experiments.*

After making a few sets of experiments, it was noticed that determinations made at different times on the same soil gave different results. Thus the following figures for the percentage lime requirement of Field 21[1] have been obtained on different occasions: 0·14; 0·15; 0·17; 0·18; expressed in lbs. per acre, the range is from 1900 lbs. to 2400 lbs., that is, the highest figure is more than 25 % greater than the lowest. It was recognised however that the determinations which failed to agree had been made under different conditions as to volume and strength of bicarbonate solution, and so it seemed necessary to make sets of determinations simultaneously on one soil under uniform conditions. Results of such are shown in Table I.

TABLE I.

Soil	Set	No.	Requirement of CaO indicated	
			Percentage	lbs. per acre
21 (before liming) ...	A	1	·143	1948
,, ,,	A	2	·151	2056
,, ,,	A	3	·143	1948
,, ,,	B	1	·179	2440
,, ,,	B	2	·179	2440
21 (4 in. deep after liming)	C	1	·156	2126
,, ,, ,	C	2	·156	2126

In sets B and C 400 c.c. of solution was used; in set A only 300 c.c. the strength of which was also rather less than in the case of the other sets.

As a further test, three samples of soil from Field 16 were treated simultaneously—A with 300 c.c. of solution of strength 0·02 N; B with 400 c.c. of the same strength; C with 400 c.c. of strength 0·014 N. The lime requirements indicated in these three cases were: A, 0·11 %; B, 0·12 %; C, 0·05 %. It seems therefore that the volume of solution may be varied within fairly wide limits without affecting the result, provided that the solution does not at any time fall below a certain concentration. This is obviously a suggestive point which merits further investigation, and is again referred to later on.

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[1] All fields mentioned herein are those of the Canterbury Agricultural College Farm.

Another point, however, must be mentioned at this stage. In one experiment a positive, though small, lime requirement was indicated for a limestone-derived soil containing about 10 % of calcium carbonate, and this suggested the possibility of a weakening of the solution (which corresponds to a lime requirement) from physical as well as from chemical causes. To test this idea, a series of trials was made. Four soil samples that had already been in contact with bicarbonate solution for 24 hours in connection with previous trials, and which were now presumably satisfied as regards their lime requirements, were filtered from their first solution and treated again with fresh solution. A sample of sand was prepared by treating alternately with concentrated HCl and strong ammonia solution, washing thoroughly, and separating a uniform sample by sedimentation. About 9 grams of this sand was treated in the same way as the soil. Another bottle contained a soil derived from limestone from Waikari; and lastly a bottle of the bicarbonate solution without any soil at all was put through the same processes as the others of this series. The results are given in Table II, the first four soils being samples which, having been previously treated, were presumably already saturated.

TABLE II.

Soil	Strength of solution	Requirement CaO indicated	
		Percentage	lbs. per acre
Nelson*	·024N	+ ·042	+572
Waikari*	·024N	+ ·025	+ 342
21 (after liming)*	·024N	+ ·042	+572
21 (before liming)*	·024N	+ ·051	+ 695
Sand	·019N	Nil	-
Weka Pass ...	·019N	− ·05	− 680
Check Bottle .	·024N	−	−
Waikari	·025N	− ·05	+ 680

    * Second treatment of sample.
    + Means a positive requirement.
    − Means that the solution has taken up lime from the soil.

These results indicate that all soils remove a certain quantity of lime from the bicarbonate solution independently of their actual lime requirement, provided that the solution is above a certain concentration initially[1]. The Weka Pass soil can scarcely be in a different chemical condition as regards lime to that from Waikari, since both contain a large excess of calcium carbonate; and yet whereas the Waikari soil

[1] This observation is confirmed by H. W. MacIntire See *American Journal of Industrial and Engineering Chemistry,* Vol. VII, No. 10 (1915), page 866.

removed lime from solution, that from Weka Pass gave it up to its solution, the strength of which was increased from 0·019 N to 0·02 N.

To return now to the point that different lime requirements are indicated for the same soil using solutions of differing strengths. The authors of the method make no suggestions as to the nature of the chemical action involved. As the soils of this farm give a strongly acid reaction to litmus, we may take it for granted that the main reaction is a simple one between the lime and the soil acids. Owing to the excess of carbon dioxide, itself a competitor for the lime, we have here a mass reaction; it will take time to complete, but when completed the result should be that the lime requirement indicated for any soil should be the same irrespective of the concentration of the solution employed, provided that the pressure in the system is kept constant. An experiment was first inaugurated to find out how long a time is necessary for the completion of the reaction. The soils used were from a sample taken 6 in. deep from Field 21, and the times of exposure increased successively by a period of 45 minutes. The initial strength of the solution was ·0216 N. The results are shown in the following Table III.

TABLE III.

Percentage lime requirement indicated after

Soil	45 m	1 h. 30 m.	2 h. 15 m	3 h	3 h 45 m	4 h 30 m	5 h 15 m.	6 h.	24 h.
Field 21	·089	131	139	146	153	153	167	·160	167

These results confirm those obtained by Hutchinson and MacLennan as showing that four hours is a sufficient length of time of exposure for practical purposes.

However to get clearer information as to the rate of progress of the reaction, one further experiment was arranged. A large Winchester was filled with 2400 c.c. of bicarbonate solution of strength ·0241 N. Into it was put 100 grams of soil from Field 21; the whole was shaken up and portions of 100 c.c. were withdrawn and titrated at intervals of 5 minutes. The method is not entirely satisfactory, as owing to the short exposure the portions were turbid and had to be filtered before titration. The results are given below (Table IV).

TABLE IV.

Percentage lime requirement indicated after

Soil	min. 5	min 10	min 15	min 20	min. 25	min. 30	min. 35	min. 40	min. 45	min. 50
Field 21	·134	·167	·191	215	215	·215	·215	·229	·229	·229

It will be noticed that with the stronger solution the end of the reaction comes much sooner than with the weaker, and furthermore (a point that has already been illustrated) the lime requirement indicated, when using a strong solution, is greater than that shown by a weak solution.   Further experimental work on this point was therefore taken in hand, ten portions of soil drawn from the same sample being treated simultaneously with solution of varying degrees of concentration.   Two series of experiments were made in this manner, the results of which are shown in tabular form below in Table V, and graphically in Figs. 1 and 2.

TABLE V.

Series A	Initial concentration of solution	·0295N	·0268N	·0246N	·0227N	·0211N
Soil from Field 21 S.S.	Lime requirement indicated as per cent. of soil	·196	·191	·161	·160	·157
		·0197N	·0184N	·0174N	·0164N	·0155N
		·143	·134	·133	·131	·120
Series B	Initial concentration of solution	·0240N	·0218N	·020N	·0185N	·0171N
Composite soil containing $CaCO_3$	Lime requirement indicated as per cent. of soil	·090	·050	·047	·048	·035
		·0150N	·0133N	·0120N	·0107N	·0096N
		·027	·013	·011	·004	0

In both these series of experiments the exposure was for a period of 4 hours.

A final series of experiments was made in duplicate   Six solutions of varying concentration were used; to ensure the completion of the reaction, the time of exposure of the soil was 24 hours; the stoppers of the bottles were placed in loosely so that the solutions were under atmospheric pressure all the time.   (This permitted the settling out of a small amount of lime as $CaCO_3$ as was proved by a control bottle without soil. but the amount so deposited was not sufficiently great to affect the general trend of the results.)   The results, which are set out in Table VI, still show the same feature -that the lime requirement indicated varies directly as the concentration of the solution.

TABLE VI.

Initial concentration of solution	·025N	·02N	·017N	·012N	·008N	·006N
Lime requirement indicated as per cent. of soil	·205	·167	·124	·073	·045	·034

From a theoretical standpoint we thus arrive at a somewhat unsatisfactory position. The authors of the method seem to have recognised this and lay down the condition that for practical purposes "the concentration of the initial solution should not be much below
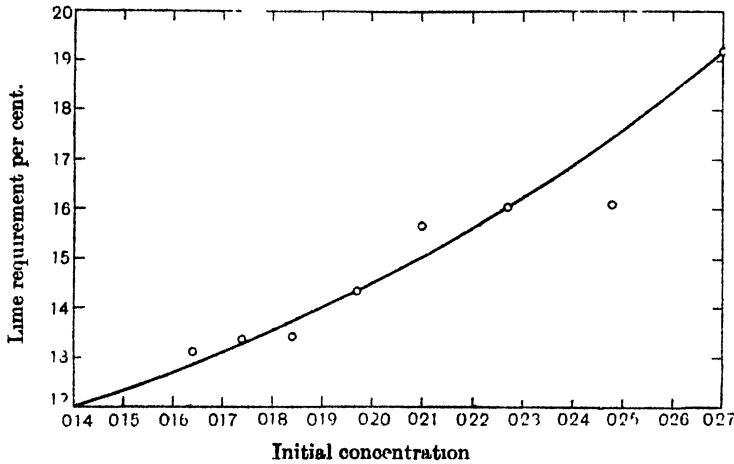
Fig 1
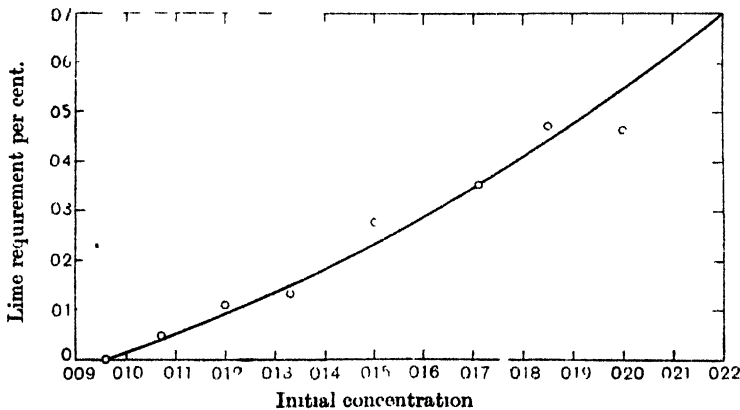
Fig. 2.

N/50 strength." They further demonstrate that using solution of this concentration and allowing 3 hours as the period of digestion their method gives results agreeing satisfactorily with the more laborious methods previously employed, such as those of Jones, of Hopkins, of Lyon and Bizzell, and of Veitch.

Adopting these standard conditions of experiment, the present writer, with a view to amplifying the practical checks on the accuracy of the method, treated two soils with known weights of calcium carbonate in suspension in known volumes of distilled water, and thereafter determined their lime requirements for comparison with those of untreated samples. The results so obtained are set out in Table VII.

TABLE VII.

The Lime Requirement of Certain Soils after Treatment with Calcium Carbonate.

Soil	Original lime requirement		Equivalent of CaO added		Lime requirement finally expected		Lime requirement actually indicated	
	Per-centage	Lbs. per acre	Per-centage	Lbs. per acre	Per-centage	Lbs. per acre	Per-centage	Lbs. per acre
21A	·136	1854	·062	845	·074	1009	·076	1037
21A	·136	1854	·118	1609	·018	245	·019	260
21B	·181	2464	·185	2520	− ·004	− 56	− ·003	− 42
21B	·181	2464	·213	2900	− ·032	− 436	− ·037	− 498

C. *Field Experiments.*

These results are satisfactory, but it is obvious a still more practical test would be to determine the lime requirements of two similar and adjacent soils, one of which had received a known dressing of lime at a sufficient length of time previously to allow of its being incorporated with the soil. To this end a number of farms were visited and samples of soil were collected from adjacent fields, one of which had been limed while the other remained untreated and so served as a check. Some of these soils are typical lime-requiring soils so far as we meet with such in this country, and the experiments are the more interesting on that account. The results are tabulated below (p. 161).

Out of these experiments the following conclusions emerge: (1) that an application of lime to a soil in the field is reflected in a diminution of the lime requirement of the soil as indicated by the method under consideration: (2) that the diminution in the indicated lime requirement is not commensurate with the amount of lime added: (3) since it has been found by practical experience that an application of 1 ton of burnt lime per acre is sufficient to convert an unhealthy infertile soil into a healthy fertile one, even in the case of the typical lime-requiring soils of Southland, then either (*a*) this method for indicating lime requirements gives excessive values or (*b*) it gives an optimum value that is greatly in excess of practical, or at all events of economical requirements.

TABLE VIII.

The Lime Requirement of some adjacent Limed and Unlimed Soils.

No.	Locality	Treatment	Lime requirement percentage	Difference from adjacent unlimed soil	
				Percentage	Lbs. per acre
L 21A	Lincoln, Canterbury	6 cwt. burnt lime, 1915	·103	·036	500
L 21B	,, ,,	No lime	·139	--	—
C 128	Ashley Dene, Canterbury	1 ton carbonate of lime, 1915	·113	·043	590
C 129	,, ,,	No lime	·156	—	—
C 219	Morven, Canterbury	1 ton burnt lime, 1913	·085	·019	260
C 220	,, ,,	No lime	·104	—	—
C 122	Longbeach, Canterbury	No lime	120	—	—
C 123	,, ,,	Limed 20 years ago	·120	nil	--
S 3	Wallacetown, Southland	No lime	·248	—	—
S 4	,, ,,	1 ton burnt lime, 1909	·226	·022	300
S 7	,, ,,	1 ton burnt lime, 1906 } 1 ton burnt lime, 1915 }	·184	·064	880
S 8	Branxholme, Southland	1 ton burnt lime. 1901	·113	·092	1250
S 9	,, ,,	No lime	·205	—	—
S 18	Edendale, Southland	2 tons burnt lime, 1896	254	·016	220
S 19	,, ,,	2 tons burnt lime, 1910	·243	·027	370
S 20	,, ,,	No lime	·270	—	—
S 21	Lochiel, Southland	8 cwt. burnt lime, 1915	·153	·068	925
S 22	,, ,,	6 cwt. burnt lime, 1914	·196	·025	340
S 23	,, ,,	Limed 20 years ago	·221	—	—
S 61	Longbush, Southland	8 cwt carbonate of lime, 1904	160	028	380
S 62	,, ,,	No lime	188	-	—
S 63	Morton Mains, Southland	30 cwt. burnt lime, 1914	·125	·083	1030
S 64	,, ,,	10 cwt carbonate of lime, 1915	·192	·016	220
S 65	,, ,,	No lime	·208		—
S 16	Woodlands, Southland	1 ton burnt lime, 1915	·159	021	290
S 17	,, ,,	No lime	·180	—	—

In connection with field experiments of this kind at the Woburn Station, Hutchinson and MacLennan have arrived at the following conclusion: "Without necessarily indicating that the controlling factor in crop production of these (barley) plots is one of physiological resistance to soil acidity, there is still a very close agreement between yields and soil reaction. In all cases where the soil is neutral in reaction, high returns are obtained; where the requirement is more than 0·18 %, the crop shows almost if not complete failure....Somewhat similar data were obtained with the soils from the permanent wheat plots, although in

this case the crop was more resistant to acid conditions, and persisted until the soil showed an absorption of over 0·22 %."

Unfortunately we have not in this country a series of experimental results bearing on this point, but practical farm methods appear to indicate that the limits suggested in the above statement are too narrow for adoption here. Thus, to take the case of the Wallacetown soil, one giving a markedly acid reaction and with an indicated lime requirement of 0·265 %, it is found that a dressing of even 1 ton and certainly of not more than 1½ tons of burnt lime, is ample for the practical purpose of putting the soil into a condition to yield an abundant harvest, while owing to the high price of lime a phenomenal return would be required for a further application to prove profitable. Another point is that the effect of liming is seen rather in the pastures than in the cereal or root crops. At Edendale (where the practice of liming was first introduced into Southland 25 years ago) they say that a want of something in the soil was seen, not so much by low yield of oats and turnips, as in the ill-health and lack of condition of stock depastured in these fields.

These points will be further referred to in the next part of this paper.

II. LIME REQUIREMENTS OF SOME SOILS OF THE SOUTH ISLAND, N.Z.

In this section the lime requirements of the soils of two South Island areas are considered, and an attempt is made to correlate the indications given by Hutchinson and MacLennan's method with the dictates of practical experience.

In order to avoid interfering factors such as differences of climate, rainfall, geological origin of the soils, systems of farming, etc., the writer decided to confine his study to two areas. Fortunately enough, the two districts most suitable from the point of view of accessibility to the writer and as having come within his own personal experience, are also entirely suitable from other considerations. These two districts are Mid-Canterbury and the Southland Plain. In the former is situated the Canterbury Agricultural College, and it is preeminently a district where the most diverse opinions are held as to the needs or otherwise of liming. In Southland the writer has fairly intimate knowledge of a good deal of agricultural country as well as a general knowledge of the whole district. What is more important is that in many parts practical experience shows that the soil *demands* lime; that,

in fact, it is impossible to farm without liming. On the other hand there are also parts, mainly the river flats, where the soils give thoroughly gratifying returns without liming and where, indeed, liming has yielded negative results. The plan of the work has been, therefore, to compare the physical and chemical characters (a) of the Southland soils that require lime with those in the same district that do not; (b) of Southland soils with those of Mid-Canterbury.

A. DESCRIPTION OF THE SOIL AREAS DEALT WITH.

1. *The Southland Plain.*

Physiography. The Southland Plain is bounded on one side by the ocean, and elsewhere by great mountain ranges that circle it round on west, north, and east. Out of these mountains the Waiau, Aparima, Oreti, and Mataura rivers have brought the loads of gravel and silt of which the plain has been built. An uplift during the formation of the plain has caused its division into two elements. (1) A low plateau, varying in elevation from 50 ft. on the seaward margin to 1000 ft. where it reaches into the mountain valleys. This plateau has been dissected into undulating country by the numerous streams consequent upon the fairly heavy rainfall. (2) A flat low-lying area fronting the plateau from its edges to the sea and including also the river flats in the lower part of their courses.

The soils of the district fall fairly well into divisions based on these structural considerations, and we have: (1) shingly soils in the inland and more elevated parts. (2) Good deep loams underlain by deep clay beds on the rest of the plateau. (3) Alluvial soils, resting on gravels or the marginal flats and in the river-beds.

System of farming As in other parts of New Zealand, the chief products of Southland are wool, frozen meat, grain, and dairy produce. The rainfall is sufficient and evenly distributed throughout the year, and consequently luxuriant pastures of rye-grass and clovers are easily obtainable; and as grass is the best and cheapest food for stock, it is not surprising that the pasture occupies an important place in the rotation. In view of the necessity for providing winter keep for the sheep and cattle, a considerable area of turnips is grown to be consumed on the ground. A typical rotation would be: 1st year, Turnips out of grass; 2nd year, Oats; 3rd year, Turnips or Rape with grass for pasture, to remain down as long as profitable, generally from 3 to 7 years.

Manures. As compared with Home conditions, the system of manuring, as with other departments of farm practice, is extensive. No farmyard manure is available except what is produced on the farm, and in only rare cases is special care taken to save that little. Artificial mixtures are universally, but sparingly, used for all crops. All of these are fundamentally phosphatic, and differ from one another in being with or without small quantities of nitrogen and potash. Many come from the freezing works, the basal portion being blood, bone, and organic refuse from the freezing industry. Considerable quantities of phosphatic guano of various grades from the Pacific Islands are used, as is also superphosphate of local, Australian, or Japanese manufacture. The average composition of the mixtures offered by eight Southland firms during the 1915 16 season is as follows:

	Sol. N percentage	Insol N percentage	Water sol. P_2O_5 percentage	Water insol. P_2O_5 percentage	K_2O percentage
Turnip ...	0·19	0 84	2 32	12 84	0 26
Grain ...	0·49	1·05	2 25	11 35	0 43
Rape and grass	0 69	0·84	1 30	11·00	0 97

The most striking point about the manurial requirements of New Zealand soils is their response to phosphates. The usual negative results from the use of potash and nitrogenous manures is due no doubt in the one case to the large reserves of potash in the soil, and in the other case partly to the richness of our soils in organic matter, and partly to the opportunities for the accumulation of nitrogen which our rotation allows with its lengthy period in permanent pasture and its turnip crops fed on the ground.

2. *The Mid-Canterbury Plain.*

Physiography This district is bounded on the north by the Waimakariri river, on the west by the foothills of the Southern Alps, on the south by the Waitaki river, and on the east by the ocean. The plain has been built up of layers of gravel, sand, and silt derived from the mountains of the west, brought down by the numerous rivers that rise therein, and spread out along the coast[1]: consequently the rivers are very rapid and their transporting power great. The average width of the plain is 30 miles, and its altitude at the margin of the foothills is 1200 ft.; that is, the gradient is 1 in 130. The average

[1] See "Formation of the Canterbury Plains" by Capt F W Hutton, F.R.S. in *Trans N Z Institute*, Vol. xxxvii, 645.

height of the river-terraces is 500 ft.[1] near the foothills, and about 50 ft. where the railway crosses them.

As would be expected from the mode of origin of the plain, every sort of soil is to be found on the surface, from rich loams overlying clay sub-soils, to thin shingly soils on gravel beds. It is not possible, therefore, to divide such a district in a thoroughly satisfactory way, into even relatively small areas, each of which shall be characterised by a soil of constant physical and chemical properties.

System of farming. What has been said of Southland under this heading applies in the main to this district also. The rotations are of the same general type, the chief differences being in the crops taken — differences which are associated with the lower rainfall and higher temperature of the northern district. Southland is noted for its heavy oat yields which sometimes exceed 100 bushels to the acre, though 55 to 60 is considered good; while Canterbury produces over two-thirds of the wheat grown in the Dominion.

B. The Question of Lime.

As already mentioned it is easy to find land in Southland that absolutely demands lime; the farmer will tell one that he cannot farm without it. The advantages to be derived from its use became generally recognised from 20 to 25 years ago, the discovery being in the nature of an accident. It had been the custom of manure merchants to tone down their mixtures with imported gypsum until, recognising that the same purpose might be served by the limestone occurring abundantly in some parts of the district, they tried this material instead. The manures so mixed were found to give specially good results, hence liming alone was tried, and with such good effects that the practice rapidly spread. The custom until the last few years was to apply "shell" lime in the Autumn at the rate of about 2 tons per acre, allow it to "slack," and cast it with shovels in Spring. preparatory to sowing down to rape and grass. To-day the practice is to sow ground burnt lime, about 1 ton to the acre, by means of a distributor of which several types are on the market. In passing, it may be mentioned that a keen discussion is raging at present as to the relative merits of carbonate of lime and burnt lime, but this point is beyond the scope of the present paper.

In certain areas, however, lime is at least not necessary and no very obvious advantages follow its use. For convenience therefore, we shall

[1] F. W. Hilgendorf: "Influence of the Earth's Rotation on the Course of the Rivers on the Canterbury Plains," *Trans. N. Z. Institute*, Vol. xxxix, 206.

confine our attention to a comparison of the soils of one such district in Southland with those of a "lime-demanding" area in the same province; and with both these we shall compare the typical Canterbury Plains soils, which, though decidedly acid in reaction, have never given a sufficiently gratifying response to liming to encourage the extended use of this material.

The most typical area of soils requiring lime in Southland includes the Plains between the Aparima and the Oreti rivers and between the Oreti and the Mataura rivers, an area of over 300,000 acres, which because of its physiographical relationship to the river flats already described, we shall hereinafter refer to as the Terrace Lands. As a suitable area of soils not requiring lime, we choose the flats of the Oreti river between Dipton and Wallacetown, an area of some 80,000 acres, which we shall refer to as the River Flats.

Lime requirement of these soils. The following tables show the lime requirements of a number of soils from these areas as determined by Hutchinson and MacLennan's method under standard conditions.

TABLE IX.

Lime Requirements of some Southland and Canterbury Soils by Hutchinson and MacLennan's method.

Group A.   Soils requiring lime from Southland Terrace Lands.

Lab. No.	Locality	L.R. %	Lab. No.	Locality	L.R. %
S 3	Wallacetown	0 25	S 41	Nightcaps	0·14
S 4	,,	0 23	S 44	Heddon Bush	0 22
S 9	Branxholme	0 21	S 45	Drummond	0 16
S 10	Wilson's Crossing	0 17	S 46	Oreti Plains	0 16
S 14	Rakahonka	0 22	S 47	Gladfield	0·17
S 15	Roslyn Bush	0 21	S 48	Sparbush	0 16
S 17	Woodlands	0·15	S 51	Aisla Bank	0·14
S 18	Edendale	0·25	S 54	The Plains	0·14
S 19	,,	0·24	S 62	Longbush	0 19
S 20	,,	0 27	S 65	Morton Mains	0 21
S 22	Lochiel	0·20	S 67	Flint's Bush	0 15
S 23	,,	0·22	S 68	Hodgkinson's	0 18
S 40	Opio	0·16	S 75	Wright's Bush	0 15
				Average	0 19

Group B.   Soils not requiring lime from Southland River Flats.

Lab. No.	Locality	L.R. %	Lab. No.	Locality	L.R. %
S 6	Wallacetown	0·15	S 36	Dipton	0·14
S 13	Tussock Creek	0·16	S 42	Wicy's Bush	0·14
S 24	Winton	0 16	S 69	Bayswater	0·14
S 25	,,	0·14	S 70	Upper Bayswater	0·14
S 26	Lady Barkly	0·14	S 71	,, ,,	0·08
S 29	Centre Bush	0·13	S 72	Lower Bayswater	0·11
S 30	Kauana	0 13	S 73	,, ,,	0·11
S 31	,,	0 12		Average	0 135

Group C. Acid but unresponsive Canterbury soils.

Lab No.	Locality	L.R. %	Lab. No.	Locality	L.R. %
	Lincoln District			*Morven District*	
L 21	Lincoln College	0·10	C 217	Willowbridge, West	0·06
C 141	Lincoln	0·14	C 218	Morven, Waihao Flat	0·09
C 142	Weedons	0 09	C 219	Morven	0·09
C 143	Weedons	0·15	C 220	,,	0·11
E 1	Ladbrooks	0 06	C 221	Willowbridge, East	0·09
E 2	Prebbleton	0·05		*Ashburton District*	
E 3	Tai Tapu	0 04			
E 4	Lincoln	0 10	C 117	Chelmsford	0·17
E 5	Greenpark	nil	C 118	,,	0·11
E 6	,,	0 04	C 119	Tinwald	0 16
E 7	Springston	0 10	C 120	Eifelton	0·09
E 8	,,	0·11	C 121	Longbeach	0·16
E 9	Gould's Road	0 09	C 122	,,	0·12
E 10	,,	0 09	C 123	,,	0·12
E 11	Lincoln	0 13	C 124	Waterton	0·12
C 129	Ashley Dene	0·15	C 125	Greenstreet	0·11
	Rakaia District		C 126	,,	0·05
C 108	Somerton	0 12	C 127	Allenton	0·09
C 109	Rakaia, West	0 11			
C 110	Rakaia, East	0 12			
C 111	Overdale	0·14			
C 112	Chertsey	0 14		Average	0 103

These figures merely confirm what has already been observed; namely, that while the Hutchinson-MacLennan method enables us to distinguish between soils *demanding* lime and soils not requiring it so urgently, yet we cannot agree to the statement that a complete failure of crop is the accompaniment of an acidity of 0·18 %. For there are Southland soils naturally requiring lime that, after a dressing of lime that experience shows to be sufficient for the practical purpose of soil amelioration, may still show as high an acidity as 0·24 %; while those of the Canterbury Plains and of the Southland River Flats, though having initially average lime requirements of 0·103 and 0·135 % respectively, have nevertheless not shown any marked demand for lime, and certainly are being farmed very profitably without it. Hutchinson and MacLennan would apparently, in the light of their experience, lime a soil till its acidity is reduced to nil; our experience, while confirming the belief that lime is *urgently* needed where the initial acidity is greater than about 0·20 %, gives us no proof from the point of view of crop production, that lime is necessary where the acidity is less than about 0·10 %.

This statement, however, should be taken strictly in the sense in which it is made. The writer does not attempt to insist that liming the soils of the Canterbury Plains, and of the Southland River Flats, will not pay; all we say is that while the benefits of liming have thrust themselves under the notice of farmers in the case of the Southland Terrace soils, they have not been sufficiently obvious to those farmers in Canterbury and in the other Southern area who have made the experiment. Nor is it necessary for the writer to say that he fully realises that a manurial dressing may be more than paying its way though the fact may not be obvious merely by viewing the plots without measurements. His conservative attitude is dictated by the considerations: first, that there is as yet no positive experimental evidence proving the economical importance of lime to these soils; secondly, that satisfactory results are being obtained without liming; thirdly, that whereas a kind of natural selection operating among methods of farming the Southland Terrace lands, brought about the evolution of the practice of liming, the same processes have not achieved similar results in the other areas under discussion.

The following evidence may be brought against the writer's attitude: (1) The process of nitrification demands the presence of a free base. "The (nitrifying) organisms will not tolerate an acid medium: a sufficient excess of calcium carbonate is therefore necessary, both in culture solutions *and in soils*[1]." (2) There is some physiological evidence, rather uncertain it is true, that lime is required by the Canterbury soils in the "depraved taste" for bones, etc., sometimes exhibited by cattle, and in the weakness in bone sometimes seen in young stock fattening rapidly on the very luxuriant spring growth on some of our heavier lands.

The bulk of the practical evidence suggests that soils in these areas having an acidity not greater than 0·10 % stand in no immediate need of liming. To translate the indications of Hutchinson and MacLennan's method into practical terms, the writer proposes to use this figure as a correcting[2] value to be deducted from the actual indication in order to get the probable practical requirement. The figure 0·10 % is selected as being the present lime requirement indicated for Field 21 of this College Farm which received 6 cwt. of lime in the winter of 1915, and which is now in an entirely satisfactory productive condition.

[1] *Soil Conditions and Plant Growth,* Dr E. J. Russell, 2nd ed (1915), p. 90.

[2] It must be remembered that the method indicates a small lime requirement even for neutral soils

III. A CONSIDERATION OF SOME POSSIBLE REASONS FOR THE
DIFFERENT LIME REQUIREMENTS OF THESE SOILS.

1. *Calcium oxide.* In trying to discover the reason for the different acidities displayed by these soils the customary appeal to chemical analysis was first made—more especially to determinations of lime—with results that are shown in Table X.

TABLE X.

Chemical Analyses of some Canterbury and Southland Soils.

Group A. Soils requiring lime—Southland Terrace Lands.

Lab. No.	Locality	Hygroscopic moisture	Ignition	Soluble in st HCl			
				CaO	MgO	P_2O_5	K_2O
S 3	Wallacetown	4·92	12·18	0·28	–	0·11	0·14
S 9	Branxholme	5 00	12·06	0·41	–	0·12	0·08
S 14	Rakahonka	5·00	11·04	0·30		0·10	0·08
S 17	Woodlands	3·71	11·71	0·48	·	0·08	0·13
S 18	Edendale	3·68	12·32	0·28		0·10	0·15
S 23	Lochiel	3 76	11·02	0·74		0·06	0·13
S 65	Morton Mains	4 10	10·92	0·54		0·09	0·17
	Average	4·31	11·61	0·433	—	0·096	0·126

Group B. Soils not requiring lime—Southland River Flats.

S 6	Wallacetown	3·76	8·38	0·65	–	0·10	0·08
S 26	Lady Barkly	3·25	8·58	1·12	—	0·06	0·19
S 29	Centre Bush	2 66	7·90	0·75		0·10	0·12
S 30	Kauana	2·85	8·93	1·01	–	0·10	0·17
S 31	,,	2·45	9·50	1·18	—	0·12	undet.
S 36	West Dipton	3·22	8·56	—	—	—	—
	Average	3 03	8·64	0·94	–	0·096	0·14

Group C. Acid but unresponsive Canterbury soils.

C 118	Chelmsford	2·48	7·90	0·64	—	0·14	0·20
C 120	Eifelton	2·52	7·23	0·70	–·	0·11	0·22
C 126	Greenstreet	2·21	6·98	0·54	—	0·13	0 14
C 141	Lincoln	2·90	8·86	0·64		0·08	0·22
L 21	Lincoln College	3 98	7·12	0·36	·	0·12	0·20
C 217	Willowbridge, West	4·21	8·59	0·58	·		0·17
C 218	Waihao Flat, Morven	4·48	9·12	0·50	—	0·17	0·23
C 220	Morven	2·70	6·44	0·59	—	0·12	0·31
C 221	Willowbridge, East	4·52	10·28	0·50		0·20	0·31
C 203	Winchester	—	—	0·41	–·	0·10	·0·33
	Average	3·32	8·06	0·546	—	0·13	0·233

Group D. Canterbury soils*: analyses by George Gray.

Lab. No.	Locality	Hygroscopic moisture	Loss on Ignition	CaO	MgO	P_2O_5	K_2O
				Soluble in st. HCl			
L 4	Lincoln College	2·48	5·46	0·15	0·30	0·14	0·17
L 17	„ „	3·43	7·12	0·31	0·83	0·31	0·20
	Springston	3·28	5·69	0·26	0·39	0·12	0·04
	Lake Ellesmere Flat	1·65	2·81	0·32	0·64	0·11	0·26
	Waikakahi	3·40	6·26	0·37	0·44	0·31	0·22
	Highbank	3·70	7·98	0·54	0·84	0·07	0·15
	Weedons	3·00	5·85	0·39	—	0·07	0·38
	Ashley Dene	2·50	6·13	0·23	—	0·16	0·10
	Hinds, Ashburton	2·40	7·62	0·86	0·83	0·28	0·38
	Average	2·87	6·10	0·38	0·61	0·17	0·21

Group E. Soils of Lincoln District. Analyses by N. M. Paulsen.

Lab. No.	Locality			CaO	MgO	P_2O_5	K_2O
E 1	Ladbrooks			0·34	0·05	0·06	0·21
E 2	Prebbleton			0·50	0·07	0·08	0·17
E 3	Tai Tapu			0·58	0·09	0·07	0·20
E 4	Lincoln			0·83	0·22	0·28	0·23
E 5	Greenpark			0·63	0·34	0·10	0·15
E 6	„	—		0·90	0·51	0·08	0·20
E 7	Springston			0·33	0·23	0·09	—
E 8	„			0·34	0·09	0·06	0·33
	Average			0 56	0·20	0·10	0·21

Group F. Determination of lime and magnesia in some Canterbury soils. B. C. Aston[1].

Locality	Sol. in st. HCl CaO	Sol. in st. HCl MgO	Locality	Sol. in st. HCl CaO	Sol. in st. HCl MgO
Woodend	0·50	0·76	Otaio	0 57	0·37
St Andrews	0·85	0·32	„	0 53	0·45
„	0·55	0·20	Studholme	0·50	0·48
Timaru	0·80	0 72	Waimate	0·45	0 30
Leeston	0·34	0 52	„	0·54	0 39
Hororata	0·30	0·51	Waitaki	0·33	0·24
Waimate	0·46	0·38	„	0·88	0·62
Orari	0·88	0·74	„	0·45	0·32
Geraldine	0·62	0·51	„	0·35	0·41
Ashburton	0·59	0·41	Geraldine	0·61	0·76
„	0·39	0·66	Pleasant Point	0·60	0·21
Waimate	0·42	0·31	Te Moano	0·36	0·57
„	0·52	0·31	Timaru	0·39	0·29
Tai Tapu	0·34	0·32	Pleasant Point	0 61	0·62
„	0·26	0·24	West Eyreton	0·28	0·49
Ashburton	0·33	0 54	Waihao Forks	0 39	0·37
			Average	0 50	0·48

* (Samples taken 8 in. to 12 in. in depth)

[1] *N Z. Journal of Agriculture*, Jan. 1916, Vol. XII, No. 1, p. 47.

In the case of the two Southland areas the explanation sought would appear to be simply in differences in the calcium oxide contents of the soils. The soils requiring lime have an average of only 0·43 % of CaO, while those not requiring lime have 0·94 % on the average. But when we come to the Canterbury soils, this explanation is insufficient, for, among ten soils analysed by the writer, having a less acidity even than the Southland soils not requiring lime, the average percentage of CaO is only 0·55. The average lime content of nine Canterbury soils analysed by Gray is 0·38 %; of eight soils of this district analysed by Paulsen 0·56 %, while 32 determinations by Aston yielded an average of 0·50 %. The variations in the average lime requirements of the soil groups shown in Table IX appear therefore to be no more correlated with differences in their lime contents than do the practical results of liming in the various areas.

2. *Calcium carbonate.* Determinations of calcium carbonate have not been made. None of these soils show marked effervescence with dilute acids, and there is no external evidence indicating a connection between lime requirement and calcium carbonate content.

3. *Lime magnesia ratio* A good deal of attention has been paid in late years to the necessity for a proper ratio of lime to magnesia in soils[1]. In this country Aston[2] has recorded definite instances of infertility due to the toxic action of excess of magnesia, and has advocated the use of lime in certain soil areas, amongst them being some Canterbury soils, for the sake of increasing the CaO : MgO ratio. His own analytical results for the Canterbury Plains soils as shown in Table X, Group F, scarcely bear out his contention in the case of these soils at least; nor do the results of Paulsen's analyses of soils of the Lincoln district, though Gray's analyses suggest a "bad" ratio. British authorities find little evidence for the theory, Russell[3] recording soils, both rich and poor, having practically identical ratios.

4. *Potash.* Nothing is revealed by the determinations of phosphoric acid and potash, for while Canterbury soils are slightly richer in the former and markedly richer in the latter constituent than Southland soils, yet the differences between the soils of the two Southland areas are small enough to be considered negligible.

5. *Hygroscopic moisture: loss on ignition and soluble humus.* An examination of Table X will show marked and regular differences

[1] For a summary see *The Plant World*, Apl. 1916, p. 83, et seq.
[2] Aston, B. C., *N. Z. Journal of Agriculture*, Vol. xi, No. 6
[3] Russell, E. J., *Soil Conditions and Plant Growth*, 2nd ed. (1915). p. 165

TABLE XI.

Mechanical Analyses of representative Soils.

Group A. Soils requiring lime—from Southland Terrace Lands.

Lab. No.	Locality	Coarse sand	Fine sand	Silt	Fine silt	Clay	Soluble humus
S 3	Wallacetown	0·8	45·1	28·6	14·8	1·7	3·1
S 9	Branxholme	2·0	40·7	29·2	16·0	1·5	3·2
S 14	Rakahonka	1·0	54·2	23·6	10·8	2·9	1·4
S 17	Woodlands	1·0	51·0	24·6	14·4	2·5	1·5
S 18	Edendale	0 4	29 4	42·3	14·9	5·1	4 4
S 20	,,	1·4	27·5	43 4	14·6	4·7	5·0
S 23	Lochiel	0 8	40·5	26·4	21·4	2·8	2·1
S 63	Woodstock	2·3	31·5	42 7	12·1	3·7	3·6
S 65	Morton Mains	1·2	26 3	41·7	19·2	4·3	2·4
	Average	1·2	38·5	33 6	15·4	3·2	3·0

Group B. Soils not requiring lime—from Southland River Flats.

S 6	Wallacetown	2 9	40 7	20 3	26 8	2 9	2·2
S 26	Lady Barkly	1·9	23 6	28·6	32·1	5 9	1·6
S 29	Centre Bush	4 3	34 4	26 4	26 1	4·1	1 4
S 30	Kauana	2 2	28 3	32 4	25 4	6 3	2 1
S 31	Dipton Flat	4 2	25 3	26·3	31 6	5·4	1 4
S 36	West Dipton	6 2	23 4	31 5	25 8	7 4	3 7
S 73	Bayswater	1 7	17·5	34 4	28 3	7 5	3 8
	Average	3·3	27 6	28 6	28 0	5 6	2 3

Group C. Soils of Canterbury Plains

C 109	Rakaia, West	2 7	50 6	26 4	12 9	2 5	1 9
C 110	Rakaia, East	1 5	46 5	31·4	14 6	2·7	1 4
C 111	Overdale	0 9	38 9	29 6	20 5	6·4	2 2
C 112	Chertsey	4 2	35 5	31·5	20·3	4 1	1 6
C 117	Chelmsford	5 7	40·2	26 6	19·3	4 4	1·9
C 118	,,	1 6	33·4	31 0	21·6	4·2	2 3
C 120	Eifelton	4 9	41 8	27 2	17·1	2 9	1 2
C 121	Longbeach	4 6	40 4	26 1	19·1	3 6	0 7
C 122	Longbeach Estate	4·8	38 3	24·3	24 6	4·0	1 6
C 124	Waterton	2 5	33·8	31 8	21·5	5·7	1 6
C 126	Greenstreet	3·3	34·4	28 8	18·7	8 2	1·8
C 114	Lincoln	2·3	36·5	36 1	14 2	4 5	3·3
L 21	Lincoln College	2 3	23·5	33·0	28 3	4·4	5·0
E 1	Ladbrooks	1 8	42·6	26·2	18 1	5 7	4·0
E 2	Prebbleton	9·3	37·6	25 4	19·5	2·3	2·6
E 3	Tai Tapu	0·6	31·3	31·6	27·0	3·6	2·1
C 127	Allenton	0·5	23·8	38·8	27·2	3·2	1·7
C 217	Willowbridge	3 3	34 9	25·9	23·3	6·7	1·5
	Average	3 2	36·9	29·5	20·4	4·4	2·1

between the percentages of hygroscopic moisture and the losses on. ignition in the soils of Groups A, B, and C. The Southland soils requiring lime have an average of 4·31 % of hygroscopic moisture and an average loss on ignition of 11·61 %; while those not requiring lime have only 3·03 % of hygroscopic moisture and 8·64 % loss on ignition. The corresponding figures for Canterbury soils sampled and analysed by the writer are 3·32 % and 8·06 %. Gray's averages for nine soils are 2·87 and 6·10, while those of 25 analyses[1] by Aston of Canterbury soils give 2·56 % of hygroscopic moisture and 6·14 % loss on ignition; while the average figures for six Southland lime-requiring soils are 4·18 % and 11·56 %.

The sharp differences in these quantities form a clear line of demarcation between soils of the Southland Terrace Lands on the one hand, and those of the Southland River Flats and of the Canterbury Plains on the other. How far the differences in lime requirement depend upon differences in the content of organic matter possessed by the various soils is a matter for further consideration, meanwhile it may be mentioned that the figures expressing the average percentage of "soluble humus" (that is, organic matter dissolved by the approximately 1 % ammonia solution preliminary to mechanical analysis) have the same general aspect; they are: for Southland Terrace Lands 3·0; for Southland River Flat soils 2·3; and for the soils of Canterbury Plains 2·1.

6. *Mechanical analyses.* In modern soil studies mechanical analysis is deemed to play an important part. The results obtained by the conventional methods of the Agricultural Education Association applied to representative soils from our three areas are shown in Table XI (page 172).

The information gleaned from a study of these mechanical analyses is not particularly suggestive. If lime were needed for the amelioration of the physical texture of the soils as conditioned by the size of their ultimate particles, we should expect that the soils of the River Flats, which are undoubtedly heavier than those of the Terrace Lands. would prove more responsive to liming.

The physical structure of these soils as revealed by mechanical composition is conveniently brought under comparison by summing into two groups the averages of the coarser fractions, namely, sand and silt, on the one hand, and the averages of the finer particles, fine silt and clay, on the other, as shown below:

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[1] Collected from various Divisional Reports and Journals of N. Z. Dept. of Agriculture.

Soil area	Average percentage of coarse sand and fine sand and silt	Average percentage of fine silt and clay
Southland Terrace Lands	73 3	18·6
Southland River Flats	59 5	33·6
Canterbury Plains	69·0	24·8

Since the coarser fractions are those that tend naturally to make soils open and friable, while the fine silt and especially the clay are affected by the action of lime, one must conclude that the response of these soils to liming is in no way connected with the amelioration of their texture, at any rate so far as the surface soil is concerned.

7. *Character of sub-soil.* The soils of which the mechanical analyses are tabulated above are samples drawn 6 in. deep, this being the depth of soil usually stirred by the plough in routine work in these districts. Up to the present time, I have not collected enough samples of the sub-soil to make possible a useful comparison, though on my collecting tours I made extensive notes of the character of the sub-soil and of the underlying formation   In general the soils of the Terrace Lands overlie deep beds of a somewhat sandy clay, exceedingly dense in texture, and almost completely impervious   These beds range from 3 ft. to 8 ft. in depth, and lie upon the old gravels mentioned on a previous page.   The soils of the River Flats are from 8 in to 2 ft. in depth and lie upon very open loose gravels of recent deposition.   There is thus a fundamental difference in the nature of these two soil areas: the soils of the River Flats are naturally exceedingly well drained and ventilated; those of the Terrace Lands tend to remain water-logged in the wet weather, for they have no under-drainage, and their mechanical constitution favouring the retention of water, they lie wet till the moisture is drawn off by the natural slope of the country.   It is not surprising therefore to learn from the practical farmer in this area that draining is an essential preliminary to liming.

Turning to the Canterbury Plains, we find here a great diversity of sub-soils, from the open gravel beds typically seen in the Burnham district to the sandy clay sub-soil met with on this College Farm.   But in comparing the retentivity for moisture of the soils of Southland and Canterbury a new factor comes into operation, namely the annual rainfall and other climatic conditions.

8. *Meteorology.* The principal items in the meteorological statistics of Southland and Canterbury are shown in the accompanying Table XII, which is abstracted from the Official Records.   More complete informa-

TABLE XII.

Meteorological Statistics.

	Shade temperatures from self-registering instruments recorded at 9 a.m. daily			Mean humidity satura-tion 100		Rainfall				Total bright sunshine in hours	
	Mean monthly maximum		Mean monthly minimum		Mean temp. for year			Total fall in inches		Number of rainy days		
Year	L.	I.	L.	I.	L.	I.	L.	I.	L.	I.	L.	I.	L.	I.
1913	62 2	57·6	43 8	41·2	53·0	49·4	66·0	80·0	31·51	58·18	139	243	1950	1514
1914	63·5	58·0	43·7	41·0	53·6	49 5	63·2	79·7	20·95	49·54	120	216	2281	1778

L. = Lincoln, Canterbury.   I. = Invercargill, Southland.

Average rainfall at Lincoln (33 years) = 25·39 inches.

Average rainfall at Invercargill (18 years) – 45·98 inches.

tion is to be found in the *New Zealand Year Book* for 1915 (Government Printer, Wellington). The figures relating to rainfall show that Southland has an annual fall almost twice as great as that of Canterbury and that it is spread over a very much larger number of days. Of course these figures are strictly true only for the two places at which the records were taken; nevertheless they reflect very faithfully the climatic differences between the two districts. Rainfall is recorded at a large number of stations scattered about the districts and a summary of their reports as published in the "Monthly Meteorological Statistics" in the *Gazette* yields the following additional information:

Rainfall for 1915.

Average of six (6) Southland Stations = 45·64 inches

.,   ten (10) Canterbury   ,,   = 17·08 ,,

It is thus apparent that the Canterbury soils enjoy (a) more moderate rainfall, (b) fewer rainy days, (c) more bright sunshine, (d) higher temperatures than those of Southland. In accordance with these conditions which favour bacterial life, no less than that of higher plants, we find that oxidation proceeds much more rapidly in Canterbury soils than in those of Southland, so that in the former there is always less soluble humus and less total organic matter (as indicated by loss on ignition) than in the latter—facts of which evidence is given in the analyses given in Tables X and XI. When we compare the soils of the two Southland areas we find that those of the River Flats which are favoured with good natural under-drainage, and consequently with

good aeration, and by inference, with higher temperatures[1], have less organic matter, soluble or otherwise, than those of the Terrace Lands.

Observations recorded by Hilgard in his volume on *Soils in the Humid and Arid Regions* illustrate the same phenomenon of the rapid oxidation of organic matter in dry soils, as the following figures gleaned from the Tables on pp. 136, 137 of the 1910 reprint of this work will show.

Average percentage of humus in 41 soils of the arid region   0·91

Average percentage of humus in 15 sub-irrigated arid soils   1·06

Average percentage of humus in 24 soils under humid conditions   ...   .   4·58

From these considerations it appears probable that the excessive acidity of the soils of the Southland Terrace Lands as compared with those of the River Flats, and with those of Canterbury, is due mainly to the lack of natural under-drainage which in this wet district causes the retention of an excessive amount of water, the presence of which retards the oxidation of organic matter and encourages the accumulation of "sour" humus

SUMMARY AND CONCLUSION.

1. The Hutchinson-MacLennan method for determining the lime requirements of soils, when practised under suitable standard conditions, gives more reliable indications than are obtainable by the ordinary methods of chemical analysis.

2. The method gives indications which appear to be uniformly in excess of the actual requirement of the soil for lime as judged by economic standards: hence a correcting value seems advisable.

3. The correcting value for the soils of Canterbury Plains is about 0·10 %.

4. The greater acidity and higher lime requirement of soils of the Southland Plains appears to be due to a combination of lack of natural

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[1] In this connection it is important to note that the only localities in Southland where wheat can be grown successfully are Bayswater in the basin of the Aparima river and the Dipton Flat of the Oreti river valley. In other parts the wheat is almost always destroyed by frost. That this is due to differences in temperature of soil and overlying air, there can be little doubt. Damage to wheat by frost is very rare in Canterbury, but last season a severe frost (10 to 14 degrees) caught the plants just when they were ready for fertilisation and totally ruined crops over areas estimated at 12,000 acres (see *N. Z. Journal of Agriculture*, January, 1916).

under-drainage and high rainfall, which prevents aeration and oxidation of organic matter, so that "sour" humus accumulates in the sòil.

In conclusion the author desires to acknowledge the assistance of his predecessor, Mr George Gray, F.C.S., who has kindly allowed him the use of many hitherto unpublished analyses; and of his students, Mr N. M. Paulsen, M.A. for assistance with the chemical analysis, and Messrs O. C. Stephens and N. P. Neal for assistance with the mechanical analysis of a great many soils.

SOME CONDITIONS AFFECTING THE VALUE OF CALCIUM CYANAMIDE AS A MANURE.

By T. DUNCAN MOSSCROP, B.Sc. (Agric.), Lond., C.D.A., Wye.

[THE following is an account of experiments carried out by the author during the years 1913-14 in collaboration with Professor S. J. M. Auld, D.Sc., Ph.D., F.I.C., etc., of University College, Reading. Dr Auld is now engaged in military duties and the task of presenting this report therefore devolves upon the author who wishes it to be clearly understood that Professor Auld must not be held responsible for any inaccuracies or other faults.]

Although calcium cyanamide has now definitely established itself as one of the nitrogenous manures no clear attempt appears to have been made to ascertain the cause of the injurious effect which it is generally recognised as having upon vegetation and germinating seeds. Hall[1] merely states that "the chief drawback to the practical employment of calcium cyanamide as a manure is its light, blow-away character, and the injurious effect upon germinating seeds of the ammonia and other gases given off when it is first applied to the soil." The first drawback has now been overcome by the introduction of granular calcium cyanamide. With the idea of settling definitely the cause and extent of injury to germinating seeds the following experiments were undertaken.

The calcium cyanamide used was of the "blow-away" description and was bought in the ordinary way from a manure merchant.

BELL-JAR EXPERIMENTS.

Twenty-five cos lettuce seeds were placed on a porous tile under each of four bell-jars which were sealed from the outer air by water. One was used as a control and had no calcium cyanamide. Each of the others had

[1] *Fertilisers and Manures*, 1909 ed p. 40.

4 gm. calcium cyanamide in a crucible, mixed in one case with water, in another with damp soil, and in the other with soil-water to ascertain whether the soil bacteria would affect the matter. In no case was the calcium cyanamide or any liquid from it allowed to come into contact with the seeds. In the control twenty-two seeds had germinated by the fifth day but those in the other bell-jars soon darkened and were black by the fourth day. Microscopic examination showed that whilst the testa (seed-coat) was uninjured the cotyledons were blackened and completely disorganised. Both the water and air in these three jars were strongly alkaline at the end of the test. Some lettuce seeds were then immersed in bench ammonia for a few hours, the same blackening and disorganisation of the cotyledons following. Fresh seeds and tiles were then used and dilute ammonia was put in the crucible. The seeds blackened in 24 hours and none germinated. Similar tests were then undertaken with one gram of calcium cyanamide and turnip and barley seeds, but apparently the robuster seeds were able to withstand the smaller quantity of calcium cyanamide as there was very little difference in the germination.

Under certain conditions—generally of storage and non-bacterial decomposition –there may be formed from calcium cyanamide, cyanamide $CN . NH_2$, dicyandiamide $C_2N_4H_4$- - a polymerisation product, and cyanamide carbonate. All of these compounds are stated to be distinctly toxic. The polymer is fairly stable and does not produce ammonia on distillation with magnesia. It is also extremely unlikely to be formed under the conditions existing in the soil. Cyanamide and its carbonate are rather different and might conceivably be formed as intermediate products. Now cyanamide itself is, when pure, a solid melting at 40° C. But it is usually separated from its solutions as a liquid and is described as volatile in steam, i.e. it has an appreciable vapour pressure. As it forms salts with alkalies it is reasonable to suppose that, if formed, it would combine with the CaO in the fertiliser, but it was deemed advisable to set the matter beyond doubt by further experiments. Accordingly ten seeds each of lettuce, swede turnip and wheat were placed on tiles under bell-jars under the following conditions:

No. 1 was the control.

No. 2 contained a crucible in which were 2 gm. of calcium cyanamide and water, the crucible being tied over with filter-paper soaked in NaOH so that if cyanamides were formed they would combine with the NaOH.

No. 3 was the same as No. 2 but the filter-paper was untreated.

No. 4 was furnished with a crucible containing 2 gm. $(NH_4)_2 SO_4$

and 2 gm. $CaCO_3$ and covered with untreated filter-paper. This was to imitate the calcium cyanamide as far as possible with the exception that the only gas which could be formed would be ammonia.

The results are set out in Table I and show clearly that ammonia is the inhibitory cause: the resistance of the seeds appears to be in direct proportion to their size.

TABLE I.

Bell-jar experiments, showing total number of seeds germinated on each day.

	1	2	3	4	5	6	7	8	9	10	11	Day
Lettuce		5	9	9	9	9	9	9	9	9	9	Control
	—										0	Calc. cyanamide + NaOH
		—								—	0	do. only
	—					1	1	1	1	1	1	Ammon. sulph. + $CaCO_3$
Swede	—		8	9	9	9	9	9	9	9	9	Control
	—			—							0	Calc. cyanamide + NaOH
											0	do. only
	—		2	2	2	2	2	2	2	2	2	Ammon. sulph. + $CaCO_3$
Wheat	—	—				2	10	10	10	10	10	Control
	—	—	—	—			—	—	—		0	Calc. cyanamide + NaOH
	—	—	—	—		—	—				0	do. only
	—	—	—	—	—		4	4	6	6	8	Ammon sulph. + $CaCO_3$

Laboratory Experiments.

A quantitative estimation of the gases evolved when calcium cyanamide is acted upon by water was made, 100 c.c. of water and 4·737 gm. calcium cyanamide being used and a week allowed for the action. The results were as follows.

CO_2—0·001183 gm. = 0·0237 % of calcium cyanamide used.

H_2C_2—trace.

NH_3—0·0204 gm. = 0·43 % of calcium cyanamide used.

The spark was then passed through the residual gases but there was no appreciable change in volume.

An estimation was also made of the NH_3 produced when soil was used in addition to water. Five grams of calcium cyanamide and 100 gm. fine soil and 100 c.c. water were used and one week was allowed. The amount of NH_3 formed was 0·1428 gm. or 2·86 % of calcium cyanamide used, *i.e.* nearly seven times as much as with water alone.

Pot experiments.

Numerous pot experiments were also undertaken with the object of demonstrating further that ammonia is the injurious substance produced and of ascertaining what length of time must elapse before the manure could be considered absolutely safe. Calcium cyanamide was used in amounts considerably higher than would be adopted in ordinary farm practice in order that the points aimed at might be more clearly demonstrated. A mixture of $(NH_4)_2SO_4$ and CaO was used at the same time to imitate as closely as possible the ammonia-producing properties of calcium cyanamide. Since granular nitrolim has now superseded the old powder form it is hardly worth while to give the figures, but it may be remarked that the mixture of sulphate of ammonia and quick-lime gave results which agreed remarkably with those from calcium cyanamide and that the injurious action had disappeared eight days after the manure had been applied to a moist soil.

It would therefore appear probable that any injurious effect on germination when calcium cyanamide is used is due to the formation of free ammonia produced at first more rapidly than it can be adsorbed by the soil.

Any danger to non-oily seeds or those with a thin testa can be avoided by applying the calcium cyanamide a week before sowing the seeds.

HYDROLYSIS OF THE SOLUBLE PROTEIN OF SWEDE TURNIPS.

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In the Agricultural Returns published by the Board of Agriculture, the figures for swedes and turnips are given together. Taking the figures for 1913, we find that swedes and turnips were grown on 1,757,000 acres of land in the United Kingdom, and that the total weight of crop obtained was 25,319,000 tons   No other green crop occupied so large an area, and no other crop of any kind produced as great a weight of fodder

Recent work on the Chemistry of Nutrition has shown that the nutritive value of a protein may be affected by the relative amounts of the individual amino-acids contained in it   The average composition of the protein eaten by an animal should, in respect of the amino-acid balance, approximate as closely as possible to that of its body proteins. If in the mixed proteins of a ration there is for instance a considerable excess or deficiency of some one amino-acid there will probably be an uneconomical utilisation of the protein as a whole.   These considerations make it highly desirable that the agriculturist should have a complete knowledge of the amino-acid content of his feeding stuffs in order to enable him to avoid protein waste in compiling rations for his stock   This research is intended to serve as a contribution to such knowledge.

The variation in the "total solids" of swede turnips has been studied by Collins[1], but the protein seems to have escaped the attention of investigators   Addyman[2] gives the following figures for the amount of protein in swede turnips

Juice   97·466 grams containing sol. albuminoids 0·167 gram
Solids   2·534   ,,   ,, insol.   ,,   0·216   ,,

100·000 grams   0·383 gram

[1] *Journ. of Agric Sci* 1, 89, 1905   [2] *Agric Analysis*, p. 113.

The present paper deals with the soluble protein contained in the juice.

PREPARATION OF THE PROTEIN.

The swedes used were fairly well-grown specimens freshly pulled up from the ground at various times during the winter; they were quite free from any signs of disease. They were cleaned and washed to remove adherent soil particles, and then passed through a shredding machine. The juice was squeezed out by means of a press, the average amount obtained being about 40 per cent. of the weight of shreds taken. The turbid juice was filtered through ordinary filters and was then obtained as a transparent slightly dark-coloured liquid. Fresh filters had to be used every other day owing to the rapid growth of acid-producing bacteria, causing precipitation of the protein before filtration. The clear filtrate was heated in beakers up to 90° C. for about half an hour, which served to precipitate all the protein as a practically white curdy mass. The precipitate was allowed to settle, and then the supernatant liquid was carefully poured off. The beakers were filled up with hot distilled water, and the protein allowed to settle again, whereupon the water was poured off as completely as possible. The protein was washed seven times with hot distilled water in this manner. It was next washed three times with alcohol by decantation, and finally ether was poured on to it. Great economy of alcohol was effected by carrying out the washing in the vessels of a large centrifuge. The ether was filtered off on a Buchner funnel, and the protein washed on the filter twice with ordinary ether, and finally with anhydrous ether   It was then placed in a vacuum desiccator over sulphuric acid; it soon dried to a light grey mass which was easily ground down to an impalpable powder in a mortar. After heating in a water oven for two or three days it ceased to lose weight.

One lot of six litres of the clear filtered juice treated as described above gave 9 grams of dry protein   This represented a yield of 0·15 gram of protein from 100 c.c. of juice.

One preparation obtained in this manner contained

> 14·09 per cent. nitrogen (determined by the Dumas and Kjeldahl methods),
> 1·81 per cent. water,
> 8·60 per cent. ash,

the percentage of nitrogen in the dry ash-free substance thus being

15·73. Another preparation gave 15·64 per cent. of nitrogen in the dry ash-free substance.

An attempt was made to purify the protein by dissolving it in 0·2 per cent. soda and reprecipitating with acid, but the protein would not dissolve. It was realised, however, that the ash present, although abnormally high, would not interfere with the hydrolysis, so the work was carried on without any further attempts being made to get rid of mineral impurities.

The ash gave qualitative tests for calcium and phosphate. An estimation of the phosphate present showed that it contained 12·05 per cent. P_2O_5 equal to 26·33 per cent. of calcium phosphate.

An estimation of the sulphur in the protein was carried out by the Benedict method as modified by Wolf and Osterberg[1].

$$0·4408 \text{ gram gave } 0·1349 \text{ gram } BaSO_4$$
$$= 0·01853 \text{ gram sulphur}$$
$$= 4·204 \text{ per cent. sulphur.}$$

This result is particularly high, much of the sulphate probably coming from the ash. The protein, however, gave a copious precipitate of lead sulphide when boiled with concentrated caustic soda solution and lead acetate, showing the presence of organic sulphur.

ARGININE, HISTIDINE, LYSINE (AND AMMONIA).

The estimations of the diamino-acids were carried out according to the familiar method of Kossel and Kutscher, as modified by Kossel and Patten, and also by Steudel.

1. *Hydrolysis and estimation of protein.*

Forty grams of the swede protein (equal to 35·96 grams dry ash-free protein) was hydrolysed by boiling under a reflux condenser with a mixture of 120 grams of concentrated sulphuric acid and 240 grams of water. The flask was first heated on a water bath for two and a half hours until solution was complete, and then in an oil bath for fifty-six hours at 105° C. The cold hydrolytic liquid was made up to 1 litre with water and two determinations of the nitrogen present in 5 c.c. made by Kjeldahl's method.

5 c.c. of hydrolytic liquid gave NH_3 equal to 20·2 c.c. N/10 acid
$$= 0·02828 \text{ grm. N.}$$
$$\text{Total N present } = 5·656 \text{ grms.}$$
$$\text{N calculated in 40 grms. protein } = 5·636 \text{ grms.}$$

[1] *Biochem. Zeit.* xxix, 429, 1910.

2. *Removal of sulphuric acid. Estimation of ammonia and humin nitrogen.*

The remaining 990 c.c. of hydrolytic liquid were transferred into a four litre flask, heated to boiling, and hot saturated baryta solution added with constant shaking until the reaction was only faintly acid, and the barium sulphate filtered off by suction. The barium sulphate was removed from the funnel, rubbed up into a cream with distilled water, boiled and filtered. Although this washing process was repeated five times, the final washing liquid was still slightly coloured, and possessed the characteristic beef-tea smell of the original filtrate. The filtrate and washings were evaporated down and made up to 1 litre. Nitrogen estimations in two portions of 5 c.c. each were now made by Kjeldahl's method.

5 c.c. of liquid gave NH_3 equal to 18·63 c.c. N/10 acid
$$= 0.02608 \text{ grm N}$$
1000 c.c. of the liquid contained *5·216 grms. N.*
N originally present was 5·656 grms.
N used in analyses was 0·0566 grm.
N left before removal of sulphuric acid is 5·656 − 0·0566
$$= 5.599 \text{ grms. N.}$$
N contained in barium sulphate
$$= 5.599 - 5.216 \text{ grms.}$$
$$= 0.383 \text{ grm.}$$

This nitrogen is usually said to be contained in the melanin which is held up by the barium sulphate. It is known as "Humin Nitrogen I."

Humin Nitrogen I -- 0·383 grm. – 6·89 per cent of total nitrogen.

Two determinations were now made of the amount of nitrogen present as ammonia by distilling portions of 100 c.c. with magnesium oxide. Before adding the magnesia the solution was made neutral by barium carbonate.

100 c.c. of liquid gave NH_3 equal to 25·05 c.c of N/10 acid
$$= 0.03507 \text{ grm. N.}$$
1000 c.c. of liquid would give *0·3507 grm. N*
$$= 0.4258 \text{ grm. } NH_3.$$

Allowing for losses due to portions used for analyses, 0·4258 grm. ammonia is contained in 35·2414 grms. protein

$$= 1.21 \text{ per cent. of ammonia.}$$

The ammonia was removed from the remaining 800 c.c. by evaporation with barium carbonate and magnesia on the water bath for two hours. The two portions freed from ammonia were combined, and the alkaline magnesia and barium carbonate mixture filtered off and thoroughly washed; the excess of barium was removed from the filtrate by dilute sulphuric acid, and the precipitate filtered off and washed out. Filtrate and washings were combined together, evaporated down and made up to 1 litre, and a Kjeldahl nitrogen determination again made. Allowing for the nitrogen given off as ammonia, the difference between this and the previous estimation gives the "Humin Nitrogen II" contained in the alkaline barium carbonate magnesia mixture.

5 c.c. gave NH_3 equal to 16·66 c.c. N/10 acid.
5 c.c. gave HN_3 equal to 16·70 c.c. N/10 acid.
   Average *16·68 c.c.*
       = 0·02335 grm. N.
  1000 c.c. contain *4·670 grm. N.*
Weight N before removal of ammonia = 5·1642 grms.
Weight N lost in ammonia   = 0·3507 grm.
 Humin Nitrogen II must be 0·1431 grm.
  Leaving   4·6704 grms.
Humin Nitrogen II = 2·77 per cent. of total nitrogen.

3. *Precipitation of arginine and histidine.*

This was carried out according to the method of Kossel and Kutscher. The solution of the bases finally obtained was made up to 1 litre; a Kjeldahl nitrogen determination in 20 c.c. gave the amount of nitrogen in the silver-baryta precipitate.

20 c.c. gave NH_3 equal to 12·40 c.c. N/10 acid.
20 c.c. gave NH_3 equal to 12·52 c.c. N/10 acid.
   Average 12·46 c.c.
     = 0·01744 grm. N.
Total nitrogen in silver-baryta precipitate = *0·8722 grm.*

4. *Estimation and isolation of histidine.*

(a) The greater portion of the histidine was removed by precipitation with mercuric sulphate. The solution was concentrated to about 250 c.c., and sulphuric acid added till the solution contained 5 per cent. of it. The solution was treated with 10 per cent. mercuric sulphate solution in 5 per cent. sulphuric acid until no further precipitate was

obtained. The yellow precipitate of histidine mercury sulphate was allowed to stand for twenty-four hours, filtered off, washed with 5 per cent. sulphuric acid, suspended in water and decomposed with hydrogen sulphide. The mercury sulphide was filtered off, washed, and the sulphuric acid removed quantitatively from the filtrate and washings by baryta. The liquid was then made up to 250 c.c., and two Kjeldahl nitrogen determinations made.

25 c.c. gave NH_3 equal to 15 c.c. N/10 acid.
25 c.c. gave NH_3 equal to 15 c.c. N/10 acid.
$$= 0.021 \text{ grm. N.}$$
250 c.c. contain   0.21 grm. N
$$= 0.775 \text{ grm. histidine.}$$
Allowing for portions removed for analysis, this weight becomes
0.833 grm. histidine.

(*b*) The filtrate from the histidine mercury sulphate was freed from mercury by hydrogen sulphide and from sulphuric acid by neutralising to litmus with baryta, and adding barium nitrate as long as a precipitate was formed. Both precipitates were filtered off and washed. The solution was then concentrated to 300 c.c., acidified with nitric acid, and treated with silver nitrate, as before, till a test drop gave a yellow colour with baryta. The solution was nearly neutralised to litmus with baryta, and then a suspension of barium carbonate was added, and the mixture warmed on the water bath[1], and finally brought to the boil on a sand bath. Upon cooling, the precipitate of histidine silver was filtered off, washed with dilute baryta water (10 drops saturated cold solution per 100 c.c.). The precipitate was then decomposed in the usual way, and the solution made up to 250 c.c.

25 c.c. gave NH_3 equal to 4.65 c.c. N/10 acid
$$= 0.00651 \text{ grm. N.}$$
250 c.c. contain   0.0651 grm. N.
0.0651 grm. N $= 0.2402$ grm. histidine.
Increasing this so as to allow for losses due to analysis, it becomes
0.2583 grm.
Total weight of histidine obtained 0.833 grm. + 0.258 grm.
$$= 1.091 \text{ grm.}$$
$$= 3.04 \text{ per cent. in the protein.}$$
The histidine was then isolated as the hydrochloride.

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[1] *Vide* Steudel, *Handb. der Biochem Arbeit.* II, 498, 1910.

5. *Estimation and isolation of arginine.*

The filtrate containing the arginine was saturated with baryta, and the precipitate of silver arginine so obtained decomposed in the usual way and the liquid made up to 1000 c.c.

50 c.c. gave NH_3 equal to 12·00 c c $N/10$ acid.

1000 c.c. would give NH_3 equal to 240 c.c. $N/10$ acid

$$= 0·336 \text{ grm. N}$$
$$= 1·044 \text{ grms. arginine.}$$

Allowing for portions removed for analysis this equals a percentage of 3·12 of arginine in the protein.

The solution was freed from sulphuric acid by baryta, and the filtrate neutralised with nitric acid and evaporated down The copper nitrate double salt was then made by dissolving the arginine nitrate in water, boiling with excess of copper carbonate, and evaporating down nearly to dryness.

6. *Estimation and isolation of lysine.*

The lysine was contained in the filtrate from the precipitate of the silver salts of arginine and histidine as obtained in (3). The barium and silver were removed by means of sulphuric acid and hydrogen sulphide respectively. The solution was evaporated down to 600 c.c. and adjusted to contain 5 per cent. of sulphuric acid The lysine was now precipitated by means of a 25 per cent. solution of phosphotungstic acid in 5 per cent. sulphuric acid. After twenty-four hours the precipitate of lysine phosphotungstate was filtered off and well washed with 5 per cent. sulphuric acid. The precipitate was then decomposed with hot saturated baryta, and the barium phosphotungstate carefully washed. The alkaline solution of lysine was treated with carbon dioxide to remove the baryta, concentrated, filtered and evaporated nearly to dryness Water was added, the barium carbonate filtered off and washed, and the filtrate and washings made up to 500 c.c.

25 c.c. gave NH_3 equal to 23·82 c.c. $N/10$ acid

$$- 0·03335 \text{ grm. N.}$$

500 c.c. would contain 0·667 grm. N.

This weight of nitrogen is equal to 3·478 grms. of lysine, or 10·07 per cent. of lysine in the protein.

All attempts to isolate lysine picrate, however, failed to produce more than enough to account for 4·35 per cent. of lysine.

The remaining solution was evaporated down to dryness, and a small quantity of alcohol added to the sticky residue. A saturated solution of picric acid in alcohol was then added, but only a small precipitate was obtained. It was allowed to stand for twenty-four hours, and the lysine picrate filtered off. More picric acid was added and more picrate filtered off. This was repeated again. The lysine mother liquor was now acidified with sulphuric acid, the picric acid extracted with ether, the lysine precipitated as phosphotungstate, and the above process for obtaining lysine picrate repeated.

Total weight of recrystallised lysine picrate obtained = 3·28 grms.

Allowing for portions removed for analysis this becomes 3·86 grms.

$$= 1·503 \text{ grms. lysine.}$$

This gives a content of lysine in the protein of 4·35 per cent.

The picrate exploded in a melting-point tube at 246° C. It was analysed both by the Kjeldahl and Dumas methods.

0·1177 grm. gave NH_3 equal to 15·75 c.c. N/10 acid

$$= 0·02205 \text{ grm. N}$$
$$= 18·74 \text{ per cent. N.}$$

Lysine picrate contains 18·67 per cent. N.

0·1039 grm. gave 17·35 c.c. Nitrogen at 21 C. and 758 mm.

$$= 0·01935 \text{ grm. nitrogen}$$
$$= 18·63 \text{ per cent. nitrogen.}$$

Lysine picrate contains 18·67 per cent. nitrogen.

GLUTAMINIC AND ASPARTIC ACIDS, GLYCINE, ALANINE, VALINE, THE LEUCINES, PHENYLALANINE AND PROLINE.

A weight of the protein equal to 288·08 grms. of the dry ash-free substance was hydrolysed by boiling with three times its weight of concentrated hydrochloric acid. The flask was first warmed on the water bath until solution was complete; it was then heated in an oil bath at a mean temperature of 118° C. under a reflux condenser for forty-four hours. At this temperature the liquid boiled fairly briskly.

Separation of humin substances.

The cooled solution was filtered through a Buchner funnel covered with linen and the black insoluble matter thoroughly washed.

Weight of dry humin bodies obtained = *13·65 grms.*   •

= 4·74 per cent. of the protein.

0·3746 grm. gave NH_3 equal to 13·3 c.c. N/10 acid.

1 grm. would give NH_3 equal to 35·6 c.c. N/10 acid
$$= 0·0498 \text{ grm. N.}$$

Total N in humin bodies is *0·68 grm.*

It also contained 6·6 per cent. of ash.

An attempt to isolate glutaminic acid as hydrochloride.

The hydrolytic liquid and washings were now concentrated *in vacuo* down to about 750 c.c. The thick liquid was poured into an enamel pot, and saturated with dry hydrochloric acid gas at 0° C. and placed in the ice chest. It was kept at 0° C. for twelve days with occasional cooling below 0° C. by means of a freezing mixture. The liquid was seeded with a few crystals of glutaminic acid hydrochloride, but no indication of a separation could be detected. 500 c.c. of ice-cold absolute alcohol were added, and the mixture filtered through a pad of asbestos. A jelly-like black residue was obtained which, however, gave no glutaminic acid hydrochloride on recrystallisation. This was put back with the filtrate.

Esterification of the amino-acids.

Foreman[1] has described a new method of converting the amino-acids into their esters, for which he claims greater efficiency than the older methods. He has carried out a hydrolysis of casein using this method, and obtained higher results for the mon-amino-acids than any previous worker. This method has been employed in this hydrolysis.

Preparation of the lead salts of the amino-acids.

The hydrolytic liquid containing the alcohol was concentrated to a syrup under reduced pressure at 40° C. Steam was now passed through this syrup to get rid of some more of the hydrochloric acid. The liquid was made up to about 1700 c.c., placed in an enamel pot, and heated up to boiling point. Steam was passed through, and a suspension of 150 grms. of freshly prepared lead hydroxide added slowly. After about twenty minutes the dark residue of undissolved lead hydroxide containing humin bodies was filtered off and thoroughly washed.

The combined filtrate and washings were poured back into the vessel, steam passed in, and lead oxide added in 50 grm. lots. About 550 grms. of lead oxide were thus added, steam being passed in all

[1] *Journ. of Agric. Sci.* IV, 431, 1911.

the time. This process took about forty minutes to complete. The liquid was only very faintly acid after the final addition of litharge. The hot liquid was filtered, and the undissolved litharge thoroughly washed. The filtrate and washings were evaporated down to dryness in an evaporating basin, the pasty residue of lead salts being stirred as long as this was possible.

Weight of undissolved lead hydroxide = 136 grms. containing
0·628 grm. of nitrogen.
Weight of undissolved lead oxide = 439 grms. containing
1·598 grms. of nitrogen.

The lead salts were finally dried at 100° C., the weight of dry lead salts obtained being *489 grms.*

Esterification of the lead salts.

The dry lead salts were placed in a large porcelain jug, and 1500 c.c. of absolute alcohol added. Dry gaseous hydrochloric acid was passed in for about four hours, and the mixture was then warmed on the water bath for half an hour. A brisk evolution of hydrogen sulphide took place at first during the passing in of the hydrochloric acid. The lead salts soon passed into solution, and a precipitate of lead chloride was formed. Finally the liquid was saturated with dry hydrochloric acid gas at 0° C. The lead chloride was filtered off (ordinary filter-paper being used) and thoroughly washed with absolute alcohol.

The weight of lead chloride was 175·7 grms. containing
3·87 grms. of nitrogen.

This high figure is due to the presence of the ammonia as ammonium chloride in the lead chloride precipitate. The brown-coloured filtrate was concentrated under reduced pressure at 40° C. By this means the greater part of the hydrochloric acid and esterification water was removed.

Removal of the remaining free hydrochloric acid.

The thick residue in the distillation flask was diluted down with absolute alcohol and cooled to 0° C. A solution of dry ammonia in absolute alcohol was then carefully added until the liquid remained only faintly acid to litmus. The ammonium chloride so formed was filtered off and thoroughly washed. In this way the hydrochloric acid was removed without any production of water which would saponify the esters and lower the yield. An alcoholic solution of the ester hydrochlorides was left.

Liberation of the esters from the ester hydrochlorides.

The filtrate and washings were evaporated down under reduced pressure at 40° C. until as much as possible of the alcohol was removed. The thick dark residue of hydrochlorides was dissolved in dry chloroform and filtered from a small insoluble residue. This residue is mainly ammonium chloride which is slightly soluble in alcohol, but insoluble in chloroform. The clear filtrate and washings were poured into a two-litre flask, 270 grams of anhydrous baryta added, and the mixture thoroughly shaken. A slight rise in temperature occurred, and after standing for twenty minutes the flask was cooled under the tap. The liquid was then filtered off, the baryta and barium chloride thoroughly washed, and the chloroform removed from the filtrate under reduced pressure at 40° C. The residue was then extracted with dry ether. It did not dissolve satisfactorily, showing that the liberation of the esters from their hydrochlorides had been incomplete. Upon adding a few drops of water to a test portion of the chloroform solution, however, and adding dry baryta, the filtrate became free from chlorides. The ester hydrochlorides were now redissolved in chloroform, 20 c.c. of water added, and then about 550 grms. of dry baryta. The baryta swelled up and became granular while the whole mixture became quite hot. The mixture was thoroughly shaken from time to time. After forty-five minutes the liquid was cooled and the baryta and barium chloride filtered off. The filtrate did not give the chloride reaction. The baryta and barium chloride mixture was thoroughly washed with dry chloroform, and the filtrate and washings evaporated down *in vacuo*. The residue dissolved readily in dry ether, only a slight deposit of dark brown pasty matter remaining adhering to the sides of the flask. The ethereal solution of the esters was allowed to stand over fused sodium sulphate for a few days. The weight of dried baryta and barium chloride obtained was 649 grms. which contained 19·5 grms. of nitrogen.

The addition of water to the ester hydrochloride as described above was quite unnecessary; it was found during the preparation of a second yield of esters from the residues of the first esterification that the addition of more baryta liberated the esters from the hydrochlorides without any addition of water (see page 191). The water added must have lowered the yield of esters considerably owing to saponification.

Foreman in his hydrolysis of casein referred to previously, only obtained 25 grms. of esters from 288 grms. of casein by the application

of this process to the residues from the first esterification, whereas in this hydrolysis nearly double that amount was obtained.

Fractional distillation of the esters.

The ethereal solution was poured off from the sodium sulphate and the ether boiled off under reduced pressure. (It would have been better to have driven off the ether at ordinary pressure, because condensation would have taken place and the distillate could have been worked up for any esters which might have distilled over.) The esters were then fractionally distilled in an apparatus similar to that described by Fischer[1].

Three fractions only were obtained, further fractionation being unnecessary. The lowest pressure was obtained by absorbing the remaining gases in an evacuated tube containing freshly prepared cocoa-nut fibre charcoal surrounded by liquid air.

Table of fractions obtained.

Fraction	Temperature of bath	Temperature in vapour	Pressure	Weight
No. 1	Up to 90° C.	Up to 65° C.	15 mm.	20·55 grms.
No. 2	90° C.—126° C.	65° C.—93° C.	15 mm.	77·65 grms.
No. 3	126° C.—176° C.	93° C.—120° C	1 mm	55·61 grms.

The liquid air condenser contained 24·1 grms. of liquid, mostly chloroform and ether. Fraction 1 also contained chloroform and ether.

The undistilled residue weighed 32 grms

Preparation of a second yield of esters.

The baryta used for liberating the esters was now treated so as to produce a second lot of esters. The undistilled residue and the substance insoluble in ether (soluble in chloroform) were also treated for the same purpose.

(i) Treatment of the baryta and barium chloride mixture.

The dry mixture was placed in a beaker and a litre of water added. It was then warmed and treated with sulphuric acid (1 to 3) until no further precipitate was obtained. The liquid was now strongly acid owing to the hydrochloric acid liberated from the barium chloride. The barium sulphate was filtered off and thoroughly washed in the usual manner. The weight of the barium sulphate was 562 grms. and it contained 2·24 grms. of nitrogen

[1] Fischer and Harries. Ber. xxxv, 2158, 1902

(ii)   Treatment of the residue in the distillation flask.

This was shaken up with water and ether to extract any phenyl alanine ester.   The aqueous extract was saponified by boiling with baryta, the barium removed with sulphuric acid, and the filtrate added to the liquid for making lead salts.

(iii)   Treatment of residue insoluble in ether (soluble in chloroform).

This was boiled with water under a reflux condenser for seven hours, acidified with sulphuric acid (up to 5 per cent.) and treated with 25 per cent. phosphotungstic acid to remove the bases.   The sulphuric and phosphotungstic acids were removed from the filtrate by means of baryta and the liquid added to the others for preparing lead salts.

Preparation of lead salts from filtrates (i), (ii), and (iii).

The lead salts were made just as described before, only the treatment with lead hydroxide was discarded.   The acidity disappeared almost completely so that much lead chloride was produced.   The dry lead salts weighed *271 grms.*   The undissolved litharge weighed 525 grms. and contained 1·54 grms of nitrogen.   The liberation of the esters from the hydrochlorides by baryta in chloroform solution was carried out quite easily, no addition of water being found necessary.

The baryta residue used weighed 740 grms. and contained *11·7 grms. of nitrogen.*   The residue of esters left after distilling off the chloroform was completely dissolved by dry ether.   The ether was distilled off as completely as possible at the ordinary pressure and the esters fractionally distilled as before.

Fractional distillation of the second yield of esters.

Fraction	Temperature of bath	Temperature in vapour	Pressure	Weight
No 1	Up to 90° C	Up to 65° C	15 mm	4 1 grms
No 2	90° C —126 C	65° C —93° C.	15 mm	15 5 grms
No 3	126° C —180° C	93° C —125° C	1 mm	13 3 grms

The liquid air condenser contained 35 grms. of liquid, mainly ether and chloroform.

The undistilled residue weighed *16·3 grms.*

Fraction 1.

The esters were immediately reconverted into their amino-acids by boiling under a reflux condenser with four times their volume of water.   The alkaline reaction, however, did not disappear, even after heating for fourteen hours.   The chloroform was distilled off at the end of

this time together with about 50 c.c. of water. The distillate was strongly alkaline and fumed with concentrated hydrochloric acid. The Fraction 1 produced in the second distillation was saponified in the same way The two liquids were then combined, evaporated down, and the acids dried in the oven until of constant weight. The weight of dry acids obtained from the two fractions was *12·3 grms.*

Fraction 2.

These esters were saponified in the usual way by diluting with four times their volume of water and boiling vigorously under a reflux condenser for eight hours. The oily drops soon disappeared and the reaction became neutral. Fraction 2 from the second distillation was treated in the same way, and then combined with the other. The weight of dry acids obtained from the two distillations (Fraction 2) was *71·6 grms.*

Proline.

The dry acids of Fractions 1 and 2 were placed in separate flasks and absolute alcohol added to them These were then warmed up and kept at boiling point for about ten minutes. After standing over night the alcohol was filtered off through ordinary filters The small amount of solid on the papers was washed back into their respective flasks by means of absolute alcohol. These extractions were repeated twice again in exactly the same manner.

The alcoholic extracts were evaporated down *in vacuo* at 40° C. In order to dry the residues in the distilling flasks thoroughly, about 20 c.c. of concentrated sulphuric acid were placed in the distillate flasks (after pouring off the alcohol which had come over) and the vacuum maintained by clipping the side tubes. Absolute alcohol was added to the dry residues, much solid remaining undissolved. The insoluble portions were filtered off and returned to their respective flasks. The solutions were now evaporated to dryness *in vacuo* as before, and the residues thoroughly dried. The dry residue from Fraction 2 went up completely into cold absolute alcohol, but the extract from Fraction 1 had to be retreated. Finally the two extracts, perfectly soluble in cold absolute alcohol, were combined and made up to 500 c.c. Kjeldahl determinations of the total nitrogen present in the solution were made.

10 c.c. gave NH_3 equal to 26·8 c.c. $N/10$ acid
$$= 0·03752 \text{ grm. N.}$$
500 c.c. contain *1·876 grms. N.*

A determination of the amino nitrogen present was now made by means of Van Slyke's apparatus. 10 c.c. of the solution were evaporated to dryness and the residue dissolved in water.

This gave 14·6 c.c. of N at 16·8° C. and 764 mm.

$$= 0·01659 \text{ grm. N.}$$

Half of this comes from the amino groups

$$= 0·00829 \text{ grm. N in amino form.}$$

The total weight of amino nitrogen is *0·415 grm.*
The proline nitrogen is 1·876 − 0·415 grm.

$$= 1·461 \text{ grms.}$$

Proline contains 12·17 per cent. of nitrogen.

$$1·461 \text{ grms. nitrogen} = 12·02 \text{ grms. of proline.}$$

This is equal to a percentage of 4·17 of proline in the protein.

Phenylalanine.

The esters of Fraction 3 were diluted with three times their volume of water and extracted three times with ether. The ethereal extract was washed three times with water, the washings being poured back to the aqueous solution. The ether was distilled off and the phenylalanine ester· poured out into an evaporating basin. This was placed on a water bath and concentrated hydrochloric acid added. (A serious loss of ester took place here owing to some violent spurting caused by adding the acid too suddenly.) The liquid was now evaporated down to dryness, a nearly white residue of phenylalanine hydrochloride being left. Fraction 3 of the second distillation was treated similarly for phenylalanine ester, but one extraction with ether was considered sufficient. The saponification with hydrochloric acid was conducted in a flask so as to avoid any risk of loss. The two yields were united.

The weight of the hydrochlorides after standing over sulphuric acid and caustic soda in a vacuum desiccator was *40·7 grms.* (The ether extract from the distillation residue (first distillation) was saponified with hydrochloric acid, and gave a black residue which was not treated further.)

The crude hydrochlorides were recrystallised from concentrated hydrochloric acid. The first crop of crystals obtained weighing 9·4 grms. was pure phenylalanine hydrochloride containing 7·02 per cent. nitrogen (theoretical 6·94 per cent). A second fraction weighing 3·9 grms. contained too high a percentage of nitrogen and had to be recrystallised.

A third fraction was obtained after treating the mother liquor with a little animal charcoal. This also was impure, and was recrystallised from water. Finally 14·5 grms. of pure hydrochloride was obtained, a large refractory mother liquor residue weighing 25 grms. being left.

0·1282 grm. phenylalanine hydrochloride gave NH_3 equal to 6·32 c.c.
N/10 acid   = 0·008848 grm. N
                          = 6·90 per cent. N.

Calculated for $C_9H_{11}O_2N . HCl$ = 6·94 per cent. N.

14·5 grms. phenylalanine hydrochloride correspond to 11·87 grms. of free phenylalanine.

A further quantity of 1·03 grms. of phenylalanine was obtained during the subsequent treatment of Fraction 3 (see page 208), making a total weight of phenylalanine of *12·90 grms.*, equal to 4·47 per cent. in the protein.

Fractional crystallisation of Fraction 1 from water.
(*After extraction of proline.*)

The acids of Fraction 1 were fractionally crystallised from water, and the melting point and nitrogen content of each fraction determined in order to obtain some indication as to their constituents.

	Weight	Melting point	Nitrogen content
Crop 1	0 9 grm	280° C.	12·14 per cent
Crop 2	3 7 grms	266° C	15·31 ,,
Crop 3	3 0 ,,	258° C	15·58 ,,
Residue	3 2 ,,	236° C	—
Total	10 8 grms		

The melting point of glycine is 240° C. (Van Rostand gives 232° C.—236° C) with decomposition, d-alanine melts with decomposition at 297° C , d-valine at 315° C , l-leucine at 297° C., and d-isoleucine at 280° C. Glycine contains 18·67 % nitrogen, alanine 15·73 %, valine 11·97 %, and the leucines 10·69 %.

The analysis of crop 1 indicates the presence of much valine. Crops 2 and 3 seem to be largely made up of alanine. The residue should contain nearly all the glycine.

Glycine.

The picrate method of Levene and Van Slyke[1] was used to isolate the glycine from the residue from the fractional crystallisation of

[1] *Journ. Biol. Chem.* XII, p. 285, 1912.

Fraction 1. The 3·2 grams of acids were dissolved in nearly four parts of hot water and 4 grams of picric acid added. The mixture was cooled to 0° C. and allowed to stand for two hours. The yellow crystals which separated were filtered off in a cooled funnel, washed once with a few c.c. of cold water, and three times with cold 95 % alcohol. The crystals thus obtained weighed 0·9 grm. and softened at 200° C., decomposing at 202° C. The filtrate was now treated with 45 c.c. of N sulphuric acid, and the picric acid extracted with ether. The sulphuric acid was then removed quantitatively by means of 130·4 c.c. of cold saturated baryta water. The barium sulphate was filtered off and the filtrate and washings evaporated down to dryness. The residue was recrystallised from water and the acids left in the mother liquor weighing 0·87 grm. treated for a further yield of glycine picrate as described above. 0·62 grm. of glycine picrate was obtained which decomposed at 202° C. Attempts were made to obtain glycine picrate from 0·5 grm. portions of crops 2 and 3, but with no success. The picric acid was removed from these test portions and the acids put back.

Ether and chloroform distillates treated for glycine

The ether distillate from the esters (second distillation) was shaken up with dilute hydrochloric acid, and the acid extract evaporated to dryness Practically no residue was obtained. The chloroform distillates however, when treated in a similar manner, gave a residue of 1·6 grms. of acid hydrochlorides. This was treated with silver sulphate solution to remove the chlorine, the excess of silver removed by hydrogen sulphide, and the sulphuric acid by baryta The weight of dry amino-acids thus obtained was 1·02 grms melting at 136° C.--110° C. and containing 14·54 % N. It evidently contained leucine or valine along with alanine and glycine. It was recrystallised from water and the more soluble part remaining in the mother liquor treated for glycine picrate. 0·280 grm. of glycine picrate was obtained equal to 0·31 grm. allowing for the portion removed for analysis.

A further small quantity (0·1 grm.) of glycine picrate was prepared from the distillate obtained during the saponification of Fraction 1 (see page 194).

The total weight of glycine picrate obtained was 1·93 grms. equal to 0·76 grm. glycine. This corresponds to a percentage of 0·27 of glycine in the protein.

Fraction 2.

The acids of this fraction left after extraction of proline were recrystallised from water in the same manner as those of Fraction 1.

	Weight	Melting point	Nitrogen content
Crop 1	24 2 grms.	277° C.	11·22 per cent
Crop 2	13 2 ,,	280° C.	12·02 ,,
Crop 3	4 5 ,,	290° C.	12·11 ,,
Crop 4	4 3 ,,	277° C.	12·83 ,,
Crop 5	4 8 ,,	258° C.	14·08 ,,
Residue in mother liquor	5 7 ,,	226° C.	14 84 ,,
Total	56 7 ,,		

Crop 1 is probably a mixture of valine and leucine. Crops 2, 3 and 4 seem to contain more and more alanine mixed with the leucine and valine. Crop 5 and the residue seem to be predominantly made up of alanine mixed with valine and perhaps a little leucine.

Separation of leucine and valine.

This was carried out by Levene and Van Slyke's lead method[1]. This method was first of all applied to the first crop obtained from Fraction 2.

0·1502 grm of the mixture on combustion gave 0·2967 grm. CO_2 and 0·1292 grm. $H_2O = 53·87 \%$ carbon and 9·55 % hydrogen.

On the assumption that only valine and leucine are present, this indicated the presence of about 70 per cent. of leucine in the mixture.

$$\frac{\text{Percentage of C in mixture} - \text{Percentage of C in valine}}{\text{Difference between percentage of C in leucine and valine}} \times 100$$

$$= \frac{53·87 - 51·28}{54·96 - 51·28} = \frac{2·59}{3·68} \times 100 = 70·4 \% \ leucine.$$

A similar calculation based on the nitrogen content of the mixture, however, indicated the presence of about 60 % of leucine in the mixture.

$$\frac{11·22 - 10·69}{1·28} \times 100 = 41·4 \text{ per cent. valine or } 58·6 \text{ per cent. leucine.}$$

It was decided to treat the mixture on a basis of 60 % of leucine first of all.

Owing to losses for analysis the acids weighed 23·22 grms. now. They were reduced to a fine powder in a mortar, and seven parts of water

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[1] *Journ. of Biol. Chem.* VI, p. 391, 1909

added. 34·8 c.c. of strong ammonia solution were added and the acids went completely into solution on heating a little. 55·7 c.c. of 1·1 m. lead acetate solution were added slowly with constant shaking from a burette. The flask was then cooled for an hour under the tap, and the white precipitate of lead leucine filtered off on a Buchner funnel. The mass was well pressed out, washed three times with 90 % alcohol, and finally with ether. It was then dried in a vacuum desiccator. 27·23 grms. of lead leucine were obtained equal to 15·28 grms. of leucine or 15·98 grms. after allowing for losses for analyses.

0·2941 grm. lead leucine gave 0·1910 grm. PbSO$_4$ = 0·1304 grm. Pb

$$= 44·35 \text{.per cent. of lead.}$$

Calculated for Pb(C$_6$H$_{12}$O$_2$N)$_2$ = 44·29 per cent. of lead.

Recovery of the valine.

The excess of lead acetate was removed from the filtrate by hydrogen sulphide, the lead sulphide filtered off, and the filtrate evaporated to dryness on the water bath. The dry residue in the basin was treated with 3 : 1 alcohol-ether mixture, and washed with it to remove ammonium acetate and acetic acid. The filtrate was evaporated down and retreated to recover the valine dissolved by the first treatment. 7·6 grms. of valine were recovered containing 11·41 % nitrogen showing that some leucine was still left in it.

15·28 + 7·6 = 22·88 grms. of amino-acids were thus recovered, a loss of only 23·22 − 22·88 = 0·34 grm. having occurred.

Separation of d-alanine and d-valine.

A method for the separation of d-alanine and d-valine has been recently published by Levene and Van Slyke[1] based on the insolubility of alanine phosphotungstate in 10 per cent. sulphuric acid in the presence of a 20 per cent. excess of phosphotungstic acid.

Purity of reagents. Because of the large amounts of lead acetate and phosphotungstic acid used in this method, the authors point out the desirability of using pure chemicals in order to avoid the contamination of the amino-acids with ash. No difficulty was found in obtaining lead acetate by recrystallisation of sufficient purity; it gave no residue after precipitation of a solution with hydrogen sulphide and evaporation of the filtrate to dryness. It was found impossible, however, to obtain phosphotungstic acid which gave no residue after precipitation with

[1] *Journ. Biol. Chem.* XVI, p. 103, 1913.

pure lead acetate and evaporation of the filtrate. (Any excess of lead acetate was removed by hydrogen sulphide.) A slight residue was invariably left. The phosphotungstic acid was purified by Winterstein's method as recommended by Levene and Van Slyke. The acid was dissolved in water, from which it was shaken out with ether, with which it formed an oily layer much heavier than water. The ether solution was washed several times with water and the ether driven off with constant stirring on the water bath. The dry residue was then ground up. The product obtained was white, non-hygroscopic, and dissolved readily in water forming a colourless solution.

Preparation of alanine and valine mixture.

Crops 2, 3 and 4 of Fraction 2 (see page 199), and Crop 1 of Fraction 1 (see page 197), all of which contain between 12 and 13 per cent. of nitrogen, were combined together and recrystallised. Two crops of 9·48 and 5·32 grms. respectively were removed, and a mother liquor residue of 6·3 grms. obtained. This residue should contain any alanine which may have been present in the mixture. To this were added all those crystal fractions which contained between 14 and 16 per cent. of nitrogen, namely Crops 2 and 3 and mother liquor residue of Fraction 1 (see page 197) and Crop 5 and mother liquor residue of Fraction 2 (see page 199). The gram of acids recovered from the distillates was also added. This mixture which weighed 25·23 grms. and contained 14·20 per cent. of nitrogen was considered to be a mixture of alanine and valine only.

Upon combustion 0·1460 grm. of the mixture gave 0·2395 grm. CO_2

$$= 44\cdot73 \text{ per cent. of carbon.}$$

Valine contains 51·28 per cent. of carbon and alanine contains 40·45 per cent. This indicated the presence of about 40 per cent. of valine and 60 per cent. of alanine in the mixture.

$$\frac{44\cdot73 - 40\cdot45}{51\cdot28 - 40\cdot45} \times 100 \quad \frac{4\cdot28}{10\cdot83} \times 100 - 39\cdot51 \text{ per cent. of valine and}$$
$$60\cdot49 \text{ per cent. of alanine.}$$

A similar calculation based on the nitrogen content of the mixture also indicated the presence of about 40 per cent. of valine.

$$\frac{14\cdot20 - 11\cdot97}{15\cdot73 - 11\cdot97} \times 100 = \frac{2\cdot23}{3\cdot76} \times 100 = 59\cdot31 \text{ per cent. of alanine and}$$
$$40\cdot69 \text{ per cent. of valine.}$$

The mixture may be looked upon as made up of 10·09 grms. of valine and 15·14 grms. of alanine. The mixture was dissolved in 353 c.c. of hot 10 per cent. sulphuric acid, 35 c.c. of acid for every gram of valine being used. 283 grms. of purified phosphotungstic acid were added, this quantity being equal to 14 grms. for every grm. of alanine present, and enough to make the solution to contain a 20 % excess of phosphotungstic acid. A precipitate was formed but this was dissolved by heating on the water bath. The flask was placed in ice in the ice chest and allowed to stand for twenty-four hours. A thick crust of crystals had formed round the sides of the flask. The supernatant solution containing the valine was poured off carefully, and the crystals dissolved by heating with a volume of 10 per cent. sulphuric acid equal to that originally used. Purified phosphotungstic acid was also dissolved in the acid in the ratio of 1 grm. for every 5 c.c. The solution was then allowed to crystallise again for twenty-four hours at 0° C. The crust of crystals was loosened by means of a glass rod and poured into a Buchner funnel, and washed with a small volume of ice-cold 20 per cent. phosphotungstic acid in 10 per cent. sulphuric acid.

Determination and isolation of the precipitated alanine.

The alanine phosphotungstate was at once dissolved in hot water, the solution given being rather turbid. The solution was made up to 2000 c.c. on cooling and an amino nitrogen determination made on 10 c.c. by Van Slyke's method.

10 c.c. of the solution gave 13·15 c.c. N at 17·5° C and 764 mm.
= 0·007655 grm. N in the amino form

The 2000 c.c. of solution contain 1·53 grms. of nitrogen. A duplicate gave exactly the same result. This means that only 9·73 grms. of alanine were present instead of 15·14 grms. as stated above.

The remainder of the alanine liquid (1980 c.c.) was heated to boiling on a water bath and 20 per cent. purified lead acetate solution added until excess was present as shown by a sulphuric acid test in a drop removed from the surface of the solution. The heavy precipitate of lead phosphotungstate and sulphate was filtered off and thoroughly washed in the usual way. The filtrate was concentrated to a volume of 500 c.c.; during this concentration a bulky precipitate separated out which was filtered off. An equal volume of 95 per cent. alcohol was now added to precipitate any lead sulphate left in solution. The mixture was allowed to stand for an hour to complete the precipitation.

A bulky precipitate (unlike lead sulphate) difficult to filter, separated out. The excess of lead was now removed by means of hydrogen sulphide The precipitate came down in a very fine state of division, and absolutely refused to filter clear. The liquid now became quite blue in colour. This must have been due to an insufficiency of lead acetate having been added leaving some phosphotungstic acid still remaining in the liquid In order to remove the blue colour and any remaining phosphotungstic acid, baryta water was added until the solution became alkaline, the precipitate filtered off and the excess of barium removed quantitatively by means of sulphuric acid

Levene and Van Slyke recommend the lead method of removing the phosphotungstic and sulphuric acids because less occlusion takes place than when barium is used In this case both lead and barium had to be used The result was that when the final filtrate was evaporated down to dryness and dried in a vacuum desiccator over sulphuric acid and caustic potash, it weighed only 3·7 grms —a yield of less than 40 per cent

Determination and isolation of the valine

The decanted filtrates and washings from the alanine phospho-tungstate were diluted down to 2000 c c and the amino nitrogen determined in 10 c c

A blank determination with 10 c c of 10 per cent sulphuric acid gave 0 9 c c N, 10 c c of solution gave 16 45 c c N at 16° C and 764 mm

$$= 15\cdot34 \text{ c c at } 0° \text{ C and } 760 \text{ mm}$$
$$— 7\cdot67 \text{ c c. from amino groups}$$

2000 c c would give 15·35 c c

$$= 1\cdot93 \text{ grams } N.$$

1·93 grms of nitrogen correspond to 16·13 grms of valine

The weight of nitrogen in the 25·23 grms of mixture taken for this separation was $25\cdot23 \times 0\cdot142$ grm $= 3\cdot58$ grms The nitrogen in the alanine and valine liquids as determined by Van Slyke's method was 1·93 + 1·53 grms = 3·46 grms A loss of 0·12 grm nitrogen had taken place during the manipulations

The total weight of alanine and valine indicated as present is 9·73 + 16·13 grms = 25·86 grms a weight 0·63 grm. more than the mixture originally taken. This discrepancy is due to the fact that some alanine is left with the valine in solution It is recommended to carry out a second separation to remove this residual alanine

The valine solution (1990 c.c.) was treated in exactly the same way as the alanine liquid, and the same difficulties were encountered. Baryta had to be used in the same way as with the alanine. The solution of valine, free from mineral acids and bases, was concentrated on the water bath until the valine began to crystallise at the surface. Three volumes of 80 per cent. acetone were then added and the mixture washed, using more 80 per cent. acetone, into a flask. This was stoppered to prevent evaporation of the acetone and allowed to stand over night while the valine crystallised out. It was then filtered off and washed with 80 per cent. acetone. 6·77 grms. of pure valine were thus obtained.

0·1150 grm. gave NH_3 equal to 9·9 c.c. N/10 acid

$$= 0·01386 \text{ grm. N}$$
$$= 12·05 \text{ per cent. nitrogen.}$$

Calculated for $C_5H_{11}NO_2 = 11·97$ per cent.

The filtrate from the valine was evaporated to dryness. This contained the alanine which had escaped precipitation by the phosphotungstic acid and the valine dissolved by the acetone-water mixture.

The residue obtained weighed 6·31 grms and contained 12·57 per cent. of nitrogen. This shows that the separation of the alanine and valine had not been complete. A second separation was not considered advisable, however, because about 40 per cent. had been lost during the separation. This separation applied once indicates the presence of 9·73 grms. of alanine in the protein. Allowing for portions removed for analysis this becomes 10·29 grms. This corresponds to a percentage of 3·58 of alanine in the protein.

Second separation of leucine and valine.

The 7·6 grms. of valine obtained from the first separation of leucine and valine was found to contain leucine (see page 200) so a second separation was necessary. To this were added the two first fractions obtained during the recrystallisation of portions to remove alanine (see page 199). The mixture was thoroughly ground up and mixed in a mortar and carbon and nitrogen determinations made.

0·1407 grm. gave 0·2750 grm. CO_2 equal to 53·30 per cent. of carbon.

Assuming that only valine and leucine were present this indicated the presence of about 45 per cent. of valine and 55 per cent. of leucine isomers in the mixture.

$$\frac{53·30 - 51·28}{54·96 - 51·28} \times 100 - \frac{2·02}{3·68} \times 100 = 54·9 \% \text{ leucines, } 45·1 \% \text{ valine.}$$

A very similar result was obtained basing the calculation on the content of nitrogen.

0·1181 grm. gave NH_3 equal to 9·5 c.c. N/10 acid

$$= 0·0133 \text{ grm. N} = 11·26 \text{ per cent. N.}$$

$$\frac{11·26 - 10·69}{11·97 - 10·69} \times 100 = \frac{·57}{1·28} \times 100 = 44·5 \text{ per cent. of valine and}$$

$$55·5 \text{ per cent. of leucine.}$$

The weight of dry acids left for the separation was 21·70 grms. The finely powdered acids were completely dissolved in 152 c.c. of water and 32 c.c. of concentrated ammonia. 48·6 c.c. of lead acetate solution (1·1 m.) were then added carefully from a burette. The precipitate was washed in the usual way. 21·17 grms. of dry lead salts were obtained and then analysed.

0·2467 grm. gave 0·1623 grm. $PbSO_4 = 0·1108$ gr. Pb = *44·91 per cent. Pb.*

0·2933 grm. gave 0·1925 grm. $PbSO_4 = 0·1314$ gr. Pb = *44·82 per cent. Pb.*

Mean of duplicates is *44·86 per cent. Pb.*

Calculated for Pb $(C_6H_{12}O_2N)_2$ *44·29 per cent. Pb.*

The content of lead in this preparation is too high, showing the presence of lead valine with the lead leucine to the extent of 20 per cent. Lead valine contains 47·14 per cent. of lead.

$$\frac{44·86 - 44·29}{47·14 - 44·29} \times 100 = \frac{·57}{2·85} \times 100 = 20\% \text{ lead valine, } 80\% \text{ lead leucine.}$$

It follows then that the 21·17 grms. of lead salts obtained were made up of 4·23 grms. lead valine and 16·94 grms. lead leucine. This weight of lead leucine is equal to 9·51 grms of free leucine, which becomes 9·98 grms. upon allowing for portions removed for analysis. Adding to this the 15·98 grms. of leucine obtained in the previous separation (see page 200) the total weight comes to *25·96 grms.*, which corresponds to a percentage of 9·01 of leucine isomers in the protein.

The valine indicated by the alanine-valine separation was 16·13 grms. (see page 203).

The 21·70 grms. of mixture upon which the second lead separation was carried out contained 21·70 − 9·51 = 12·19 grms. of valine. After allowing for material removed for numerous carbon and nitrogen determinations, this is equal to 12·55 grms., making a total of 16·13 + 12·55 = 28·68 grms. of valine in the protein.

The total weight of the dry acids of Fractions 1 and 2 after extraction of proline was 56·7 + 10·8 = 67·5 grms. From this 25·96 grms. of leucine, 10·29 grms. of alanine, and 0·764 grm. of glycine have been

separated, making a total weight of 37·014 grms. This leaves a weight of 67·5 − 37·014 = 30·486 grms. for the weight of the valine. The weight of acids unaccounted for is 30·486 − 28·68 = *1·806 grms.* This loss was caused probably by the large number of recrystallisations which were necessary for the satisfactory separation of the amino-acids. 28·68 grms. of valine correspond to a percentage of

$$\frac{28·68}{288·08} - 9·95 \text{ of valine in the protein.}$$

Fraction 3. Aspartic acid.

The esters, after extraction of phenyl alanine ester, were saponified by boiling for eight hours on a sand bath under a reflux condenser with 80 grms. of baryta.

The small quantity of esters of Fraction 3 produced in the second distillation was treated in the same way, and the liquids united. The liquid was allowed to stand for several days to allow the barium salt of racemic aspartic acid to crystallise out. A large amount of baryta crystallised out as well. The crystals were filtered off and washed with cold water. They were then placed in a large volume of water, heated up on the water bath, and the baryta removed by means of excess of sulphuric acid. The barium sulphate was filtered off and carefully washed and the excess of sulphuric acid quantitatively removed from the filtrate and washings by baryta. The barium sulphate was again filtered off, washed, and the filtrate and washings evaporated to dryness on the water bath. The residue was slightly coloured, but the colour was easily removed by extraction with 10 c.c. of cold water.

The crystals were filtered off, washed, dried, and weighed 4·58 grms. equal to a percentage of

$$\frac{4·58}{288·08} - 1·59 \text{ of aspartic acid in the protein.}$$

0·1533 grm. gave NH_3 equal to 11·6 c.c. N/10 acid
$$= 0·01624 \text{ gram N}$$
$$= 10·59 \text{ per cent. N.}$$
Calculated for $C_4H_7NO_4 = 10·53$.

Treatment of the filtrate from the barium aspartate.
(Fraction 3.)

The usual method adopted with the filtrate is to remove the barium quantitatively with sulphuric acid, concentrate the solution *in vacuo,*

and then to saturate it with hydrochloric acid gas in order to obtain glutaminic acid hydrochloride. The yield obtained in this manner is always very unsatisfactory,—much more of the hydrochloride can be separated before esterification. A new method, worked out by Foreman[1], was employed for the treatment of the filtrate from the barium aspartate, which was carried out as follows: The alkaline solution was concentrated *in vacuo* down to 200 c.c., and alcohol added until no further precipitate was obtained. This precipitate consists mainly of the barium salts of glutaminic and aspartic acids (only the racemic aspartic acid is removed by the barium). The precipitate was filtered off, washed with alcohol, and the barium removed quantitatively by means of sulphuric acid. The filtrate was strongly acid, showing the presence of either glutaminic or aspartic acids or both. Cold aqueous phosphotungstic acid solution was now added to the concentrated filtrate to remove basic impurities. Excess of baryta was added to the filtrate to remove excess of phosphotungstic acid, and the excess of baryta removed quantitatively by sulphuric acid. The clear filtrate was evaporated to dryness and the residue extracted with cold glacial acetic acid. This removes further impurities. The residue thus obtained weighed 4·1 grms.

0·1520 grm. gave 13·3 c.c. nitrogen at 15° C. and 762·4 mm.

$$= 0·01573 \text{ grm. N}$$
$$= 10·35 \text{ per cent. N.}$$

Glutaminic acid contains 9·53 per cent. N, and aspartic acid 10·53 per cent. The substance obtained must be practically all aspartic acid; it gave the pyrrole reaction, however, when heated with zinc dust, and tested with a pine shaving, showing the presence of some glutaminic acid. Calculating the proportion of glutaminic acid present from the N content, we find that 18 per cent. should be present.

$$\frac{10·35 - 9·53}{10·53 - 9·53} \times 100 \quad \frac{·82}{1} \times 100 \quad \frac{82 \% \text{ aspartic acid,}}{18 \% \text{ glutaminic acid.}}$$

The 4·10 grms. obtained may be looked upon as made up of 0·74 grm. of glutaminic acid and 3·36 grms. of aspartic acid. This brings the percentage of aspartic acid in the protein up to 2·75 and gives 0·26 per cent. as the number for glutaminic acid.

The alcoholic filtrate from the precipitate of barium salts was evaporated down *in vacuo*, taken up in water, and the barium removed by addition of excess of sulphuric acid. The barium sulphate was

filtered off and the excess of sulphuric acid removed by baryta. The clear filtrate was concentrated on the water bath and gave a strong alkaline reaction. Cold aqueous phosphotungstic acid was now added until no further precipitate was obtained. The precipitate was filtered off, the clear filtrate freed from phosphotungstic acid by baryta and excess of baryta removed by sulphuric acid. On concentrating the filtrate a crop of crystals separated out. These were filtered off and weighed 1·03 grms. Upon treatment with animal charcoal and recrystallisation 0·1100 grm. of it gave NH_3 equal to 6·65 c.c. N/10 acid

$$= 0·00931 \text{ gram N}$$
$$= 8·46 \text{ per cent. N.}$$

It gave the xanthoproteic reaction and also the odour of phenylacetaldehyde on heating with potassium bichromate and sulphuric acid. Phenylalanine contains 8·49 per cent. N. The substance which had separated out must have been phenylalanine.

ASPARTIC AND GLUTAMINIC ACIDS.

The yields of glutaminic and aspartic acids obtained in this hydrolysis were so unusually low that it was considered advisable to find whether better yields could not be obtained by the application of Foreman's method to the hydrolytic liquid direct. This method has already been referred to in this paper, it was applied to the filtrate from the insoluble barium aspartate of Fraction 3.

When this research was in progress the method had not yet been published and the following determinations were carried out under Mr Foreman's direction and with his kind co-operation.

Hydrolysis of the protein.

A weight of protein equal to 32·90 grms. of the dry ash-free substance was hydrolysed by boiling with 120 c.c. of concentrated hydrochloric acid under a reflux condenser for forty-eight hours. The hydrolytic liquid was concentrated under reduced pressure in order to remove most of the hydrochloric acid.

Treatment with calcium oxide. Preparation of calcium salts.

The cold residue was diluted with about 200 c.c. of water. 18 grms. of pure lime were placed in a litre flask and slaked with 100 c.c. of distilled water. The hydrolytic liquid was added to this slowly with constant cooling. The flask was then corked and shaken up for thirty

minutes. The mixture was then filtered, the filtrate being quite clear and only slightly coloured. The black residue on the filter composed of undissolved slaked lime and humin bodies was thoroughly washed. The filtrate and washings were evaporated down under reduced pressure at 40° C.

Precipitation of the calcium salts of aspartic and glutaminic acids.

The concentrated residue of the calcium salts of the amino-acids was diluted to a mobile liquid with distilled water, about 100 c.c. being used. Alcohol (98 per cent.) was now added carefully with constant shaking. A precipitate was at once formed, a little gummy matter adhering to the bottom of the flask. Alcohol was added until no further precipitate was produced. This was then filtered off and thoroughly washed with alcohol.

Treatment of the precipitated calcium salts.

The white precipitate was dissolved in water (any particles remaining in the flask were also dissolved out in water) and the calcium removed quantitatively by means of oxalic acid solution. The clear filtrate was then treated with silver sulphate solution in order to remove traces of hydrochloric acid; the excess of silver was removed by hydrogen sulphide. The solution was concentrated and cold aqueous phospho-tungstic acid solution added until no further precipitate was produced. This removes some bases precipitated by alcohol along with the calcium aspartate and glutaminate. The excess of phosphotungstic acid and the sulphuric acid (from the silver sulphate) were removed quantitatively by means of baryta. The clear filtrate, free from mineral acids and bases, was then evaporated down to dryness. The dry residue weighed *7·923 grms.*

Extraction with acetic acid.

The dry residue was ground up to a fine powder and extracted with cold glacial acetic acid. The acetic acid removes a gummy impurity which contains practically no amino nitrogen. The dry residue insoluble in acetic acid weighed 3·342 grms. This residue was white and consisted of a mixture of glutaminic and aspartic acids. A combustion of this substance was now made.

0·1236 grm. gave 0·1697 grm. CO_2 and 0·0643 grm. H_2O .
$$= 37·44 \text{ per cent. C and } 5·78 \text{ per cent. } H_2.$$

A Dumas nitrogen determination was also made.

0·1409 grm. gave 12·0 c.c. N at 14·5° C. and 769 mm.

$$= 11·4 \text{ c.c. at N.T.P.}$$
$$= 10·18 \text{ per cent. N.}$$

Analyses of aspartic and glutaminic acids and of the mixture:

Aspartic acid	*Mixture*	*Glutaminic acid*
36·09 per cent. C	37·44 per cent. C	40·81 per cent. C
5·27 per cent. H_2	5·67 per cent. H_2	6·12 per cent. H_2
10·52 per cent. N_2	10·18 per cent. N_2	9·52 per cent. N_2

Calculating the proportions of the acids present in the mixture from the *carbon content*

$$\frac{37·44 - 36·09}{40·81 - 36·09} \times 100 = \frac{1·35}{4·72} \times 100 = \begin{array}{l} 28·60\% \text{ glutaminic acid,} \\ 71·40\% \text{ aspartic acid.} \end{array}$$

Calculating on the *nitrogen content*

$$\frac{10·18 - 9·52}{10·52 - 9·52} \times 100 = \frac{·66}{1} \times 100 = \begin{array}{l} 66\% \text{ aspartic acid,} \\ 34\% \text{ glutaminic acid.} \end{array}$$

Taking the mean of the two we get 31·3 per cent. of glutaminic acid and 68·7 per cent of aspartic acid. The mixture was therefore made up of 1·046 grms. of glutaminic acid and 2·296 grms. of aspartic acid. This means percentages of $\frac{1·046}{32·90} \times 100 = 3·18$ of glutaminic acid, and $\frac{2·296}{32·90} \times 100 = 6·98$ of aspartic acid in the protein

SEPARATION OF GLUTAMINIC AND ASPARTIC ACIDS.

Preparation of copper aspartate from the mixture.

2·727 grms. of the mixture were dissolved in 200 c.c. of water and excess of copper carbonate added. The mixture was boiled for ten minutes and the excess of copper carbonate filtered off and washed with boiling water. The filtrate and washings were evaporated down until crystallisation commenced. A large crop of the characteristic needles of copper aspartate separated out over night. These were mixed with a small amount of small prisms characteristic of copper glutaminate. These were much heavier than the copper aspartate crystals and thus rendered complete separation by elutriation quite easy.

The copper aspartate crystals were air dried for a week, and weighed

2·4765 grms. equal to 1·48 grms. of aspartic acid.   0·5072 gram of the air-dried copper aspartate gave 0·1460 grm. CuO

$$= 0·1165 \text{ grm. Cu}$$
$$= 22·98 \text{ per cent. Cu.}$$

Calculated for $C_4H_5NO_4Cu . 4\tfrac{1}{2} H_2O = 23·07$ per cent. Cu.

The heavier crystals separated by elutriation were dried in the oven and weighed *0·6728 grm*. These were ground up finely and about 100 c.c. of water added. Upon warming, the crystals dissolved partly; the copper was then removed by means of hydrogen sulphide. The filtrate was evaporated to dryness and the residue of glutaminic acid weighed *0·4066 grm.*

0·1488 grm. gave 12·1 c.c. N at 15° C. and 768 mm.
$$= 0·01439 \text{ grm. N}$$
$$= 9·66 \text{ per cent. N.}$$

. Calculated for $C_5H_9NO_4 = 9·53$ per cent. N.

ISOLATION OF GLUTAMINIC ACID AS HYDROCHLORIDE FROM THE FILTRATE FROM THE COPPER SALTS.

No attempt was made to obtain a second crop of crystals from the filtrate from the copper salts   The copper was removed from the mother liquor by means of hydrogen sulphide and the liquid evaporated to dryness. The residue weighed 0·841 grm   This was a mixture of glutaminic and aspartic acids left behind in solution as their copper salts. The residue was dissolved in 1·5 c.c. of strong hydrochloric acid, boiled for a minute and then saturated with dry hydrochloric acid gas at 0° C. A heavy crop of glutaminic acid hydrochloride separated out. The liquid was allowed to stand over night in ice. An equal volume of ice-cold absolute alcohol was then added and the hydrochloride filtered off through ordinary filter-paper. It was washed with an ice-cold mixture of equal parts of saturated hydrochloric acid and absolute alcohol. The hydrochloride was dried in the oven and weighed 0·5544 grm. equal to 0·444 grm. glutaminic acid.

0·1591 grm. gave 10·2 c c N at 15·5° C. and 760 mm.
$$\doteq 0·01199 \text{ grm. N}$$
$$\doteq 7·54 \text{ per cent. N.}$$

Calculated for $C_5H_9NO_4HCl = 7·62$ per cent. N.

2·727 grms. of mixture had been taken. Calculating on the basis of 31·3 per cent. of glutaminic acid previously determined, this should

contain 0·850 grm. of that acid and 1·877 grms..of aspartic acid. 0·4066 grm. of glutaminic acid was recovered from the copper salt and 0·443 grm. obtained as the hydrochloride, making a total weight of 0·8506 of glutaminic acid. Thus all the glutaminic acid had been separated from the mixture. The aspartic acid separated as the copper salt weighed 1·48 grms. The remainder of the aspartic acid was contained in the mother liquor from the glutaminic hydrochloride, and its weight was 0·841 grm. — 0·443 grm. = 0·397 grm. The total weight of aspartic acid recovered is 1·48 + 0·397 = 1·877 grms. Thus all the aspartic acid had also been isolated from the mixture.

Tyrosine.

The filtrate and washings from the precipitate of calcium salts were evaporated down. The residue had been allowed to stand for three weeks under alcohol, during which time some insoluble substances had separated out. These were filtered off. The calcium was removed quantitatively by oxalic acid and the filtrate and washings made up tô a litre. 250 c.c. of this were taken as a test portion, made neutral to litmus by addition of caustic soda solution (its acidity was due to the hydrochloric acid liberated by the removal of the calcium) and concentrated on the water bath to crystallising point. Upon cooling a crop of crystals was removed. This was redissolved in water, boiled with animal charcoal and recrystallised.

Weight of tyrosine obtained = 0·11 grm

= 0·44 grm. in the litre.

The insoluble substances filtered off were found to give the Millon reaction, so they were treated for tyrosine. They were dissolved in water and the calcium removed quantitatively by oxalic acid. The filtrate was decolourised by animal charcoal and concentrated. Two crops of crystals were removed weighing 0·78 grm. The crude tyrosine was recrystallised from dilute ammonia. The pure tyrosine obtained weighed 0·52 grm.

0·1448 grm. gave NH_3 equal to 7·9 c.c. N/10 acid

= 0·01106 grm. nitrogen

= 7·64 per cent. nitrogen.

Calculated for $C_9H_{11}NO_3$ = 7·73 per cent. nitrogen.

The total weight of tyrosine obtained was 0·52 + 0·44 = ·96 grm. This indicated a percentage of $\frac{0·96}{32·90} \times 100 = 2·92$ of tyrosine in the protein.

Tryptophan.

The protein gave the glyoxylic reaction showing the presence of tryptophan.

Cystine.

The protein gave the sulphur reaction with strong soda and lead acetate, showing that cystine is probably present.

SUMMARY.

The results of the hydrolysis are given below:

		PAGE
Glycine	0·27 per cent.	198
Alanine	3·58 ,,	204
Valine	9·95 ,,	206
Leucine and Isoleucine	9·01 ,,	205
Phenylalanine	4·47 ,,	197
Tyrosine	2·92 ,,	212
Cystine	present	213
Proline	4·17 per cent	196
Aspartic acid	6·98 ,,	210
Glutaminic acid	3·18 ,,	210
Tryptophan	present	213
Arginine	3·12 per cent.	188
Histidine	3·04 ,,	187
Lysine	4·35 ,,	188
Ammonia	1·21 ,,	185
Humin bodies	4·74 ,,	189
	60·99	

Remarks.

Levene and Van Slyke's method for the separation of alanine and valine seems to be a valuable one. When pure chemicals are used, pure alanine and pure valine can be obtained. Pure valine only was obtained in this case, the alanine being contaminated with ash owing to the impurity of the phosphotungstic acid used. The greatest drawback is the large amount of acids which is lost in the precipitates of lead sulphate and phosphotungstate. The fact that the approximate composition of the mixture must be determined before the application of the method also militates against its usefulness. This method,

together with the picrate method for separating glycine and alanine, and the lead method for separating valine and the leucines, make it possible now to determine these five amino-acids with a fair degree of accuracy.

All the plant proteins for which hydrolyses have been published have been prepared from seeds; tuberin[1] seems to be the only protein yet prepared from a vegetative organ, and no hydrolysis has been published for it. Osborne has pointed out[2] that as these seed proteins appear externally and are lost to the plant and can take no further part in the metabolism, they are to be regarded as excretory substances. These reserve or excretory proteins, whether of plants or of animals, often yield a very high percentage of one or more amino-acids on hydrolysis. Thus gliadin of wheat and hordein of barley yield over 36 per cent. of glutaminic acid, while silk fibroin yields 36 per cent. of glycine and 21 per cent. of alanine. The alcohol soluble seed proteins yield the basic amino-acids in very small amount; lysine indeed is almost invariably absent.

No such wide differences have been found among the physiologically active proteins, the protamines excepted. They are always more equally balanced in their amino-acid content.

The protein which has been studied here has been prepared from a vegetative organ, and must be regarded as physiologically active. It shows no excessive content of any particular amino-acid and all the usual "Bausteine" are present.

Practically all the vegetable proteins already studied contain from 10 to 50 per cent. of glutaminic acid; in this case we have only 3·18 per cent. Nearly all protein hydrolyses give much more glutaminic acid than aspartic acid; in this case we find twice as much aspartic acid as glutaminic acid.

It will be interesting to see how far the figures given at present for the dibasic acids will have to be altered when Foreman's new method is applied to some of the well-known proteins. The poor yield of aspartic acid obtained by the ester method is well known; Hopkins and Savory[3] obtained much more aspartic acid by direct precipitation from Bence-Jones' protein than by the ester method. The difficulty of obtaining any glutaminic acid as the hydrochloride when less than 10 per cent. is present is also well known. These imperfections in the methods of

[1] Osborne and Campbell. ' *J. Amer. Chem. Soc.* 1896, xviii, 575
[2] Monographs on Biochemistry. *The Vegetable Proteins*, p. 9.
[3] *Journ. Physiol.* xlii, 189, 1911.

protein hydrolysis with regard to these two acids are overcome by Foreman's precipitation method.

The content of valine is unusually high, but not as high as that found by Foreman in linseed protein.

The ammonia content is low which is in harmony with the low content of dibasic acids.

The most serious loss in the hydrolysis was caused by the large mother liquor residue left by the phenylalanine (see page 197); this amounts to about 8 per cent. of the protein Another smaller loss was caused by the substances containing amino nitrogen contained in the proline (see page 196): this amounts to nearly 2 per cent.

It may be suggested that the low content of glutaminic acid in the soluble protein of swedes and the high content of that amino-acid in the proteins of cereals make these two feeding stuffs suitable for feeding together.

THE CELERY-ROT BACILLUS[1].

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In previous papers (46, 47) it has been shown that the celery plant is susceptible to bacterial attack, producing in the affected tissues a brown soft-rot which in serious cases may be so pronounced that on lifting the crop a high percentage of the plants is found to be quite worthless[2]. From such rotting material a *Bacillus* was isolated and this, when introduced into the tissues of healthy celery plants, readily induced in the inoculated organs a rapid decay similar to that observed on naturally infected plants. An account of inoculation experiments performed on celery plants with a description of the behaviour of the organism when grown on artificially prepared media has already appeared, and further observations concerning its pathogenesis in living plants and its development on certain culture media are recorded in the present paper.

1. PATHOGENESIS OF THE CELERY-ROT ORGANISM.

Inoculation of Celery Plants. Numerous inoculations from pure cultures have been performed on celery plants, the usual procedure being as follows:—A streak culture of the bacillus is made on celery-extract agar[3] and incubated at 26° C. After 24 hours there is a creamy-white growth along the streak and a little of this is transferred by means of a flamed platinum loop to the upper end of a petiole of

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[1] The present paper is an abridgment of a Thesis (presented March 1, 1916) approved for the Degree of Master of Science in the University of London.

[2] See Table in *Jour. South-Eastern Agric. Coll.* No. XXII, 1913, p. 461, or Reprint from same, p. 75.

[3] The preparation of this culture medium is described in *Jour. Agric. Sci.* Vol. VI. footnote, p. 212.

the plant to be inoculated; a single prick is then made with a sterile steel needle passing through the mass of bacteria and piercing the leaf-stalk. The inoculations have been made on young plants growing in pots and on older plants brought from the garden and placed with their roots in water. When the inoculated petioles contain a relatively large proportion of turgid parenchymatous cells, as in the case of leaves of rapidly growing young plants or the blanched inner leaves of the older ones, the rot makes rapid progress and the infected leaf usually collapses from above the point of inoculation in from 24 to 48 hours by the conversion of the tissues in the neighbourhood of the wound into a soft translucent brown pulp. Older leaves that have attained their maximum size are less susceptible and in these the inoculations frequently gave negative results; young plants that have been grown under comparatively dry conditions are also resistant. Control leaves pricked with sterile needles invariably gave negative results.

Young celery plants were also successfully inoculated by puncturing the laminae of the leaves which were then sprayed with bacteria taken from a pure culture and diffused in sterilized distilled water, the resultant bacterial suspension being applied to the leaves by means of an atomizer. Translucent water-soaked spots up to 1·2 cm. in diameter appeared within 48 hours; these were irregular in outline, being more or less angular and limited by the larger veins. In some cases the leaflets were gradually destroyed, in others the veins prevented the extension of the rot.

In order to ascertain what crops should not be grown on soil where diseased celery has been, inoculations were made on plants other than celery with results as described below.

Action of the Organism on Slices cut from healthy Roots and Tubers.

The root or tuber was soaked for two minutes in 0·1 % aqueous solution of mercuric chloride, then dropped into sterilized tap-water and finally washed over with sterilized distilled water. The slices were cut with a razor that was kept in 94 % alcohol until required, when the alcohol was burnt off by passing the razor through a bunsen flame. As each slice was cut it was dropped into a sterile petri dish and cut into halves with a sterile scalpel. One half of each slice was then inoculated from a pure culture, the other half being kept as control.

The organism readily produced a soft rot on slices of radish, potato, carrot, artichoke, turnip and swede. On slices of parsnip and mangel-

wurzel a slight discoloration was produced round the point of·inoculation but there was no definite rot.

Action on whole Roots and Tubers. The bacilli were transferred from a streak culture with a platinum loop to the spots selected for inoculation and two or three pricks were made through the bacterial slime with sterile needles; control spots were punctured without the application of the bacteria. The results were as follows:—

Radishes. Slight discoloration round the inoculated wounds but no definite rot, though after eleven days round one point of inoculation there was a whitish slime containing numerous bacteria.

Potatoes. Slight discoloration at points of inoculation but no rot.

Carrots. Slight discoloration on the fifth day after which the rot progressed from each point of inoculation and after eleven days from the commencement of the experiment the affected areas (dark brown in colour) round the four inoculated spots were from 0·6 to 2·7 cm. in diameter.

Artichokes. A blackening of the tissues immediately round the inoculated spots (but not round the controls) was noticed after a few days but no rot had set in after eleven days.

Parsnips. Slight browning round the inoculated punctures but no rot.

Turnips. Readily attacked; at the end of eight days almost wholly destroyed.

Swedes. These proved more resistant than turnips, for though the tissues in the immediate neighbourhood of the inoculated punctures were destroyed, lateral extension of the rot was soon arrested, and it proceeded inward only to about the same depth to which the needle had penetrated.

Mangel-wurzels. Appeared quite resistant, the only perceptible change being a "bleeding" at the wounds which however occurred to the same extent also at the control spots.

Inoculation Experiments on Growing Plants. Leaves of plants growing in pots of rape, turnip, parsley, carrot, parsnip, radish, swede, cabbage and broccoli were inoculated. In one experiment the plants were kept in a greenhouse, in another they were placed in the open. In nearly every instance inoculation with the bacillus resulted in an increase in the size of the wound, usually accompanied by a longitudinal splitting, but the leaves were not destroyed except in parsley and carrot, though the rot made some progress in one leaf of broccoli and one of swede.

The failure to obtain definite positive results in the majority of the plants was probably largely due to the persistent dry weather which obtained at the time the plants were under observation; in the case of parsley however all the inoculated leaves collapsed in less than 48 hours.

In another series of experiments plants were taken into the laboratory and placed under bell-jars after inoculation, thus maintaining a moist atmosphere; under these conditions they proved to be generally less resistant. In those cases where the plants were small enough two of each kind were placed under the same bell-jar, one for inoculation the other to act as control; the control plants were punctured with a sterile needle. The results were as follows:—

Turnip. All the inoculated leaves collapsed within 48 hours after treatment. Within a fortnight the crown of the plant was destroyed so that even those leaves which had not been inoculated were attacked at the base and also collapsed. The control plant though grown under the same bell-jar remained quite healthy (Plate I, fig. 3).

Inoculations made on the root of a young turnip growing under a bell-jar led to a gradual development of the rot; after 5 days it had extended from 0·15 to 0·75 cm. from the punctures. A control plant showed no evidence of rot.

Swede. Rot extended a few millimetres from the points of inoculation, then further development appeared to be inhibited and the leaves remained upright.

Lettuce. Inoculations made on lettuce plants on three separate occasions invariably gave negative results, the only difference that could be observed between the inoculated and the control leaves was that after 24 hours the former showed a dark rim round each puncture, the control wounds remaining colourless. Fourteen leaves were inoculated in all but no rot could be detected in any one of them in spite of the fact that in the third experiment a plant with young tender leaves was specially selected for inoculation.

Radish. Four leaves inoculated on the midrib all showed discoloration round the wounds after 24 hours and two of the leaves collapsed within 48 hours.

Parsley. Of three leaves treated two collapsed within 24 hours and later the third followed. At the end of a week the rot had extended to the base of the youngest leaf inoculated.

Carrot. All the inoculated leaves collapsed within 24 hours and by the fifth day had become quite withered. A control plant under the same bell-jar remained healthy.

Broccoli. The youngest of the four leaves inoculated collapsed on the third day; the rot failed to make any decided progress on the older leaves.

Cauliflower. The three youngest leaves collapsed within three days while two older ones proved resistant.

Rape. Inoculation led to a slight discoloration and splitting of the tissues at the punctures but no soft rot set in.

Cabbage. Two of four leaves inoculated collapsed in the course of a few days, the other two only showed a discoloration of the tissues round the wounds.

Parsnip. In one experiment this plant gave negative results. On repeating the experiment and inoculating two very young growing leaves of each of two plants, all four leaves collapsed within 24 hours.

Potato. Tubers were planted in pots and when the shoots were a few inches high inoculations were made directly on the haulm itself at about two inches above the ground level. Of six shoots so treated one collapsed on the fifth day after inoculation and a portion (4 cm. long) of the haulm was destroyed by the typical soft rot (Fig. 2). In the others the wounds increased a little in size but the shoots remained upright. Wounded controls showed no change.

In a second experiment with potato shoots a modification was introduced in that one pot was covered, for two days previous to inoculation, with a bell-jar which was replaced immediately after its temporary removal for the inoculations, and since the soil was kept damp an atmosphere saturated with moisture was maintained. The second pot had been uncovered from the time the tubers were planted and remained uncovered after the shoots had been inoculated. Four haulms were inoculated and four were left as controls in each pot, with the following result.

Pot A (saturated atmosphere). On the third day after inoculation two of the shoots collapsed and after two more days a third; in the fourth shoot the rot failed to make much progress although the wound increased in size and developed into a longitudinal fissure. The control shoots remained healthy and fourteen days after commencing the experiment the punctures were represented in each case merely by a small scar.

Pot B (dry atmosphere). In 24 hours the inoculations had caused a slight discoloration round the wounds, the affected areas being from 1 to 3 mm. long; although these developed later into

fissures 0·5 cm. to 3 cm. in length, these cracks were bordered by a fringe of desiccated tissue and there was no evidence of a soft rot or even of further splitting to the end of the experiment.

These results are interesting from the fact that Erwin F. Smith records[1] failure to obtain positive infection results from experiments in which *Bacillus carotovorus* was inoculated into green potato shoots.

The organism appears to be unable to attack uninjured living organs. In one experiment young plants of celery, parsley and carrot, some of which were quite sound so far as could be observed and free from aphides, others bearing a considerable number of aphides, were sprayed (using an atomizer) with an emulsion made by placing the product of a streak culture, 24 hours old, in water which had previously been sterilized. The plants were then kept in a glass case to maintain a damp atmosphere, but no rot set in on any of the plants, though, as shown earlier in this paper, young celery plants, the laminae of which had been punctured previously to similar treatment, became infected. In another experiment a young turnip plant growing in a pot was watered and placed under a bell-jar. On the following day the leaves were fringed with drops of water which had issued from the water pores around the margins of the laminae. Bacilli were transferred to a number of these drops by removing, with a platinum loop, droplets of the turbid liquid (water of condensation containing the bacteria) at the base of a streak culture and bringing these droplets in contact with the drops of water on one of the leaves, care being taken not to touch the leaf itself thus avoiding a rupture of the epidermal cells. No signs of rot appeared so evidently the bacillus was unable to attack through the water-pores. In this way the celery-rot bacillus differs from the " Black-Rot " organism (*Pseudomonas campestris*), for the latter readily produces infection through water-pores as has been proved by Erwin F. Smith (37)[2].

Middle lamella destroyed. If a little of the bacteria-containing pulp from a diseased celery plant be placed in a tube of water and shaken, the cells readily separate from one another, and when a drop of the resulting turbid liquid is examined microscopically numerous isolated cells are to be found, indicating that the organism attacks primarily the middle lamella.

It has been shown by Potter (28, 29), Spieckermann (40), Harrison (12), van Hall (9) and Jones (17, 18), that the soft-rot organisms produce an

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[1] *Bacteria in Relation to Plant Diseases*, Vol. II. p. 96.

[2] See also *Bacteria in Relation to Plant Diseases*, Vol. II. pp. 54 64.

enzyme which dissolves the middle lamella of the parenchymatous cells of fleshy tissues. Each of the investigators mentioned has prepared the enzyme free from the living organism and finds that its action on the middle lamella is similar to that of the organism itself. Potter also found that the organism (*Pseudomonas destructans*) with which he worked was able to penetrate the cell-wall and so enter the cell-cavity; though Jones considers that "this probably is to be regarded as due to physical pressure rather than to solution of membrane," Potter maintains [31] that his own interpretation is the correct one and that "there is certainly invasion of the cell-cavities by the bacilli." Again Erwin Smith states [39] that "*Pseudomonas campestris* is capable not only of destroying the middle lamella, but also of dissolving the cell-wall proper."

It is well recognised that there are bacteria which do possess the power to ferment pure cellulose, as shown by Omelianski [26], while Kellerman and his co-workers [19] give a list of twenty-one species of bacteria which cause fermentation of cellulose. It would appear however that a typical soft-rot organism[1] as exemplified by Jones's *Bacillus carotovorus* is unable to penetrate the unruptured cell-wall. Van Hall writes "Der *Bacillus omnivorus*[1] ist nicht im stande, die Zellwand zu durchbohren, und ein Eindringen in die Zellen findet also auch niemals statt," and the observations of Jones are confirmatory of this statement.

When a particle of the pulp of a celery petiole inoculated from a pure culture is taken a short distance behind the line where the bacteria are encroaching on the living tissue and subjected to microscopic examination the parenchyma is found in process of disintegration. Some of the cells are seen to be free but others may still cohere in longitudinal series while numerous bacilli are to be observed in active motion. On focussing downwards and confining one's attention to a single cell a layer of bacteria will come into view apparently extending across the whole width of the cell. As the observer continues to apply the focussing screw in the same direction the bacterial layer disappears from view but presently another layer of bacteria comes into focus. The bacteria are applied to the walls of the cell and the two bacterial layers correspond to the upper and lower walls of the cell. That the bacteria are on the outer side of the wall is shown by the fact that when one of the layers is sharply in focus, motile rods, also in focus, swim into the field of view and out again beyond the limits of the cell. When

[1] Harding and Morse consider *B. omnivorus* to be identical with *B. carotovorus* Jones.

the centre of the cell is in focus no bacteria at all are to be seen within the four lines representing the walls in optical section.

In microtome sections of fixed material from inoculated celery petioles the bacteria are found between the cells and, by accumulating in the space formerly occupied by the middle lamella, they tend to force them apart (Fig. 10). In such sections also it sometimes appears as though bacteria are within the cell-cavities; this appearance is to be interpreted as denoting that that portion of the section includes a cell-wall which is parallel to the plane of the section, the bacteria occupying the plane of the middle lamella (Fig. 11). These bacterial patches are not of frequent occurrence and do not appear in the same place in successive sections, both facts being evidence that the above interpretation is the correct one.

The material for these sections was obtained from celery petioles inoculated from pure cultures. Carnoy's Fluid was found to be useful for fixing the bacteria *in situ*; acetic alcohol was also used but this produced considerable distortion of the cell-walls. Heidenhain's Haematoxylin followed (after differentiating to remove the haematoxylin from the cell-wall while leaving the bacteria still stained) by Bismarck Brown as a counter stain gave excellent contrast, the bacilli being black or bluish black and the cell-walls a yellowish brown.

No swelling or lamination of the cell-wall has been observed in celery as has been described by Potter in infected turnips and by Jones in the carrot.

Although so many species of plants are susceptible to infection by the celery-rot organism it does not follow that the cultivation of all these plants must be avoided on soil in which an infected crop has grown in the previous season. The plants which are most liable to be attacked are those producing fleshy organs underground, particularly biennials whose roots when fully developed contain a high percentage of water; it would be undesirable therefore to grow root crops on infected soil. Plants in which the more parenchymatous organs are raised above ground (*e.g.* cabbage, cauliflower, etc.) are less readily affected in nature. The custom of growing celery in trenches with the subsequent "earthing-up" to promote blanching renders this plant when growing under those conditions less resistant to soft-rot, for not only are the young inner leaves etiolated, with a feeble development of mechanical tissue, but in addition their growth takes place·in an atmosphere that is almost continually moist.

Wrapping celery plants with a paper covering, as is sometimes

practised in lieu of "earthing-up," tends to protect them not only from mechanical injury caused by the chafing action of the soil particles but also from the attacks of snails and slugs which are frequently found gnawing the petioles. These animals might not only carry the soft-rot organism from one plant to another but would produce the wound necessary for the bacillus to gain access to the parenchyma cells. That phytophagous creatures spread infection by transferring pathogenic germs from plant to plant is inferred by Johnson and Adams in their paper on Bacterial Rot in Turnips (14) where they say that "so far as at present known, the disease gains entrance into the 'bulb' through wounds caused by slugs or grubs living underground." Erwin Smith (37) by actual experiment obtained infections by allowing snails (*Agriolimax agrestis*) taken from plants affected by *Pseudomonas campestris* to gnaw healthy plants, the snails being left in contact with the latter for one night only.

II. The Action of Antiseptics.

Nutrient Bouillon as Culture Medium. Tubes of nutrient bouillon containing antiseptics were inoculated from pure cultures and examined from day to day for signs of growth as indicated by the development of turbidity in the medium, followed by sedimentation.

Method. 9 c.c. of peptonized bouillon (+ 10 Fuller's scale) were run into each of a number of test tubes which were then plugged and sterilized. To each was then added 1 c.c. of the antiseptic in sufficient concentration to produce the required percentage in the 10 c.c. now contained in the tube. The "stock" solutions of the various substances used were made up in stoppered bottles several hours previous to their use so that the probability is that they were quite sterile when the dilutions were prepared. Lower concentrations were obtained from a "stock" solution by diluting with sterile distilled water in sterile test tubes using graduated pipettes which had previously been plugged at the upper end and then sterilized.

The necessary alcohol solutions were prepared from "absolute" alcohol (99·5 %). Formaldehyde solutions were obtained by taking one part of Schering's Formalin (40 %) and adding 39 parts distilled water, thus producing a 1 % "stock" solution. The chloroform was added directly by means of a pipette. Thymol was weighed out and added directly, after increasing the 9 c.c. bouillon to 10 c.c. by the addition of 1 c.c. sterile water. Benzoic acid and salicylic acid were

prepared in saturated solution in bouillon and the required dilutions made with bouillon.

In each of the two series of experiments four control tubes reserved for comparison in the degrees of turbidity and sedimentation, were prepared by adding 1 c.c. sterilized water to 9 c.c. bouillon in each tube. Two of the tubes were inoculated, the other two left uninoculated; as the latter remained quite clear throughout the course of the experiments they were evidence that the method employed was satisfactory for the preparation of such tubes free from outside contamination. The tubes were kept at room temperature (18°—19° C.) throughout the experiment.

Permanganate of Potash. This is unsuitable for experiments in which bouillon is used, for with 0·1 % the permanganate settled in a dense mass leaving a colourless liquid above in which motile rods were to be found; concentrations of 0·01 % and 0·001 % appeared to have no inhibitory action whatever on the development of the organism.

Copper Sulphate. A dense blue precipitate was formed on adding to the bouillon a solution of copper sulphate sufficient to produce a concentration of 0·2 %; no growth however occurred. 0·1 % checked growth and the medium remained clear for several days but it did not wholly prevent development. At concentrations of 0·01 % and 0·001 % there was no evidence that growth was affected in any way.

Mercuric Chloride. 0·01 % was sufficient to prevent development; 0·005 just failed to do so but had considerable retarding influence on development, since the medium showed no signs of turbidity until after the sixth day. Even with only 0·001 % no growth could be detected until the third day.

Carbolic Acid. 0·2 % sufficed to prevent growth while 0·1 % caused retardation.

Lysol. There was no growth with 1 % and very slight with 0·1 %; 0·05 % retarded development but 0·01 % had no appreciable effect.

Formaldehyde. In 0·01 % growth was inhibited completely; 0·005 % and 0·001 % retarded development slightly.

Chloroform. 10 % failed to prevent growth unless the tubes were shaken, but 1 % sufficed to inhibit growth if agitated at frequent intervals during the first six hours.

Benzoic Acid. There was no growth in bouillon half saturated with benzoic acid but development occurred when quarter saturated.

Salicylic Acid. When at a concentration one-fifth saturated growth was inhibited.

Thymol. The thymol used was in the form of small particles, the largest being about 1 mm. in diameter; when agitated as little as possible most of these remained floating on the surface of the medium, but when shaken they immediately sank. It was found that 0·1 % even without shaking was sufficient to prevent development.

Toluene. 1 % when shaken up in the bouillon inhibited growth. When unshaken no growth was apparent with 1 % or 2 % so long as a film of toluene remained on the surface, but after the evaporation of the film growth proceeded normally; with 4 % however no growth occurred even after the evaporation of the film.

Alcohol[1]. With 1 % growth, so far as can be observed, is unaffected, while in 2 % it is slightly retarded (when compared with inoculated control tubes), for although there is evidence of growth at the end of 24 hours turbidity is hardly noticeable and there is no sediment, while in 1 % and in the inoculated controls turbidity is more distinct and a little sediment is thrown down. In the case of 5 % no change was observed up to the sixth day after inoculation; later however the medium became turbid and a distinct sediment was formed This suggests that when the medium contains 5 % alcohol the organism is not killed but that development is in abeyance until favourable conditions supervene. As the alcohol evaporated at a more rapid rate than the water of the culture medium a point would eventually be reached at which the amount of alcohol present was not inhibitory to the organism.

Another experiment was then performed the result of which supported this view. Tubes prepared as in the previous experiment contained respectively 4 %, 5 %, 6 % and 7 % alcohol and were inoculated from a pure culture on Jan. 26th, 1915. The tubes were examined daily and all remained clear for five days; on the sixth day the tube containing 4 % showed the first signs of turbidity. Growth then commenced in the other tubes in succession with a few days' interval between the first appearance of turbidity in any one tube and in the next of the series as here shown:—

Bouillon containing	Date of inoculation	Date of first appearance of turbidity
4 % alcohol	Jan. 26th	Feb. 1st
5 % ,,	,, ,,	Feb. 3rd
6 % ,,	,, ,,	Feb. 15th
7 % ,,	,, ,,	March 1st

[1] As the results obtained with alcohol appear to be of special interest they are given in detail.

A tube containing bouillon with 10 % alcohol was kept for over two months after inoculation but no growth occurred.

The organism then is capable of resisting 7 % alcohol and can remain quiescent for some five weeks during which period the percentage of alcohol is gradually decreasing from 7 to about 2 (at 2 % as shown above the period of retardation is almost reduced to zero).

In those tubes where the development of the bacillus was markedly retarded (*i.e.* 4 % and upwards) it was found, on subjecting drops of the turbid liquid to microscopic examination, that normal rods were rarely if ever to be observed and they were represented by forms which were almost isodiametric, thus resembling cocci; rods approaching in length the typical form were seen to be constricted in the middle as in diplococci and obviously corresponded to two rods just before fission. Reference to these coccus-like forms of the bacillus is made later in this paper, but in the present connection it is to be remarked that when first observed they suggested that perhaps contamination had occurred during the preparation and inoculation of the tubes or, what was very improbable, that the absolute alcohol employed contained resistant spores which were stimulated to germination and propagation when the alcohol was at a suitable low concentration. To test this point six tubes of bouillon were prepared under aseptic conditions as before, two containing 5 % alcohol, two with 2 % and two others with 1 %. One of each pair was inoculated from a pure culture of the bacillus, the others kept as control tubes. The inoculated tubes containing 2 % and 1 % alcohol respectively showed evidence of growth on the following day, the former with a trace of turbidity, the latter distinctly turbid, and later both produced a dense sediment of rods intermediate in size between normal rods and the coccus forms. The inoculated tube containing 5 % alcohol remained clear for eight days then became turbid and eventually this also produced a dense sediment consisting however of the coccus and diplococcus-like forms. The three uninoculated tubes remained quite clear throughout the experiment, thus proving that the organism present in the inoculated tubes was not an extraneous one and confirming the conclusion that the precautions taken in preparing the tubes ensured non-contamination.

A Synthetic Solution as Culture·Medium.

For comparison with the results obtained with bouillon it was thought desirable to start another series of experiments with antiseptics using a medium of known composition.

The medium used consisted of the following:—

Distilled water	1000 c.c.
Ammonium nitrate	10·0 gm.
Dipotassium phosphate	2·5 „
Magnesium sulphate	0·4 „
Sodium chloride	2·5 „
Sodium asparaginate	3·0 „
Saccharose	10·0 „

To maintain a constant known temperature the tubes were placed in a thermostat at 25° C.

The various antiseptics were prepared and added as before; the lysol as used on the previous occasions was not available so a "Lysol Substitute" was used in its place. Control tubes (uninoculated) in which the antiseptics were added at the lowest concentration used in the inoculation remained clear throughout the experiment (white flocculi appeared in the tube containing lysol substitute but the liquid in which they floated remained clear). The results showed no wide divergence from those obtained when bouillon was the medium used.

Copper sulphate and permanganate of potash as before were inefficient in concentrations up to 0·1 %. At this strength the permanganate was thrown out of solution by the formation of an insoluble precipitate; the copper sulphate however remained in solution so higher concentrations were used, *e g.* 0·15 % and 0·2 %, but in these crystals of a copper compound made their appearance, the liquid became turbid and motile rods were present.

Formaldehyde and chloroform appear to be a little more effective in preventing growth in the synthetic medium than in bouillon.

In the tubes containing alcohol there was the same sequence in the appearance of visible signs of growth but the periods intervening between the commencement of development in tubes of 2 %, 4 % and 6 % were considerably reduced as was to be expected by reason of the higher temperature.

Benzoic and salicylic acids were prepared in saturated solutions in the medium at the temperature of the thermostat and diluted with the medium in the proportions required. Under these conditions salicylic acid as a one-tenth saturated solution prevented growth while a one fifth saturated solution was necessary with benzoic acid.

Germicides in Celery Extract.

In an endeavour to obtain information relative to the efficacy of the antiseptics in controlling the disease, trials were made to test the germicidal action of some of them. The poisonous properties of mercuric chloride and the relatively great bulk of alcohol, chloroform and some others preclude the use of these in the practical application to preventive measures. The results obtained in the preceding experiments with copper sulphate compared unfavourably with other results; when however instead of the culture media then employed an extract of celery is used its efficiency is increased since it was found on trial that 0·1 % copper sulphate in this medium inhibited growth completely. Copper sulphate therefore was selected for further trial; others were permanganate of potash, carbolic acid, lysol substitute and formaldehyde. Celery extract was the medium used in order to obtain cultural conditions more nearly approaching those obtained in the host plant.

In a preliminary experiment tubes of celery extract were prepared, each containing 9 c.c. of the sterile solution, and inoculated. At the end of 18 hours the tubes were distinctly turbid, thus giving evidence that vigorous growth was in progress; the germicides were then added as in the previous experiments to give the proportions as shown in the table. The tubes were shaken and a loopful (2 mm. loop) of the liquid in each tube was transferred to a tube of sterile celery extract at intervals of (1) one hour, (2) 24 hours, (3) three days after the treatment with the germicide, those cases in which growth occurred in the submoculated tubes are marked | and those which remained sterile −.

Germicide	percentage	Transfer at the end of		
		1 hour	24 hours	3 days
Copper sulphate	0 1	−		
Potassium permanganate	0 1		+	+
Carbolic acid	0 2	+	I	−
Lysol substitute	0·2	+	⊦	−
Formaldehyde	0 01	−	−	−
,,	0 005	+	⊦	−

The results show that copper sulphate at 0·1 % and formaldehyde at 0·01 % are very germicidal, probably all the bacilli being killed within an hour. The action of carbolic acid and lysol substitute at 0·2 % and formaldehyde at 0·005 % is slow and requires more than 24 hours to kill the organism. Permanganate of potash at 0·1 % on the other hand is rapid in its primary action but apparently the subsequent

precipitation which occurs interferes with its germicidal properties; at the end of an hour the majority of the bacilli must have been killed, for a 2 mm. loopful contained no living forms, as shown by the absence of any growth in the sub-inoculated tube. The few surviving rods, developing after the precipitation of the reagent, rendered succeeding inoculations capable of producing turbidity in the culture medium.

Copper sulphate when employed in the form of Bordeaux mixture is very efficient in controlling "Celery Leaf-blight" (35) and its success as a germicide in celery extract suggests that the spraying operations against the "blight" might tend to reduce the chances of infection by the soft-rot organism, though it is to be noted that in the Bordeaux mixture itself there is no *free* copper sulphate.

The other four reagents could be used as disinfectants in those places where celery or roots (carrots, turnips, etc.) are to be stored, if the soft-rot had been observed on vegetables stored there previously. Formaldehyde, though a powerful disinfectant, is not to be recommended however for use in this manner owing to the irritation produced on the mucous membrane by the gas when it passes out of solution.

In order to obtain confirmation of the previous results with copper sulphate and permanganate of potash the experiment with these substances was repeated and lower concentrations were also tried for comparison, with the results here shown.

		Transfer at end of			
		1 hour	4 hours	24 hours	3 days
Copper sulphate	0 1	−	−	−	−
„　　„	0 05	−	−	−	−
„　　„	0 01	−	−	−	−
Potassium permanganate	0 1	−	-	−	+
„　　　„	0 05	−			+
„　　　„	0 01	−	+	+	−

On this occasion the agitation of the tubes immediately after adding the reagent was more thorough than in the previous experiment and this probably accounts for the more efficient action of the permanganate.

A further trial was made with potassium permanganate at a concentration of 0·01 % using the celery extract at half the original strength; there was still some precipitation but the action was more effective. The tube was well shaken on adding the reagent and again immediately before each sub-inoculation. These sub-inoculations as before were made after 1 hour, 4 hours, 24 hours, and 3 days respectively, two tubes being inoculated at the end of each period:—

Inoculation after 1 hour: one tube remained clear, the other became turbid.

Inoculation after 4 hours: one tube remained clear, the other became turbid.

Inoculation after 24 hours: both tubes remained clear.

,,   ,,   3 days: ,,   ,,   ,,   ,,

III. PLEOMORPHISM.

In a former paper (46) the present writer stated that the celery-rot organism when in vigorous growth measured 2·5—3·5 ⋅ 0·6—0·7 μ, with double rods to 6·5 μ in length; occasionally rods up to 16·5 μ in length were seen. A microscopic examination of the cultures treated with the antiseptics as described in the preceding pages showed that in some cases there was considerable divergence from the normal dimensions. Observations showed that the behaviour of the organism in a culture medium may be modified in one of two directions and varies with the antiseptic with which the medium is treated. In the one case the tendency is towards an inhibition of the process by which the individual rod, on reaching a maximum length of about 6 μ under favourable conditions, divides transversely to form two rods which then separate. The result of the lapse of this fission is that the rod increases in length until it may be described as filamentous. In the other case fission occurs while the rod is still short and the organism then resembles a coccus.

The celery rot bacillus readily produces filaments in the presence of carbolic acid, lysol, "lysol substitute," benzoic acid and salicylic acid but only when the concentration is such that, while it does not cause the further development of the organism to be suspended altogether, it has the tendency not only to reduce the rate of growth but also to behave as a factor limiting the amount of bacterial substance ultimately formed. Thus filaments were generally to be found in those tubes where the sediment was less bulky than in inoculated control tubes.

That the filamentous forms were derived from the bacillus introduced at inoculation and were not the result of contamination was clearly shown in the experiment in which tubes of bouillon were prepared containing benzoic acid at three different concentrations, the dilutions being obtained by mixing a saturated solution of the acid in bouillon with sterile untreated bouillon; the uninoculated tubes even'

at the lower concentrations remained sterile while the inoculated tubes containing the acid at one-quarter and one-fifth saturation produced filaments.

The shorter filaments (to about 40μ in length) were often motile, with slow sinuous movements, but distinctly translocatory.

The longest filaments were formed in the tubes containing the synthetic medium treated with "lysol substitute" at concentrations of 0·1 % and 0·05 %. These threads were so long and intercoiled that they formed a felted mass so that the length of the individual threads could not be determined; some showing free ends however measured from 300μ to 500μ along the free portion before they became entangled in the rest and they must have been considerably longer than that. Permanent preparations were obtained by removing the felt-like sediment from the tube with a hooked platinum needle and teasing it out in distilled water; portions were transferred to cover-glasses, dried in the thermostat, fixed by passing through a flame, and stained with Heidenhain's haematoxylin (Fig. 8).

In the synthetic medium containing 6 % alcohol, filaments produced within a week after inoculation were 140μ in length, often repeatedly geniculate and locally swollen, becoming at times almost nodulose; the largest of these "nodules" were more or less spherical and $6·5\mu$ in diameter (Fig. 9).

It would appear that in some cases filament development is induced almost immediately after the bacillus comes in contact with the injurious substance; thus in the synthetic medium with 0·05 % carbolic acid there were numerous filaments within 48 hours, while, on the other hand, in the same medium but with 4 % alcohol filaments were not to be found at the end of a week from the time growth commenced but were present after three weeks.

The filaments are evidently a pathological condition brought about by adverse circumstances, for they are produced in the presence of certain antiseptics when these are at a concentration approaching that which inhibits growth altogether. A proof of their pathological nature is that portions of the felted sediment produced under the influence of "lysol substitute" were placed in a hanging drop of celery extract without development of any kind occurring; sub-inoculations were also made from the tubes containing 0·1 % and 0·05 % respectively of "lysol substitute," a drop of the liquid containing particles of the sediment being in each case transferred to a tube of the untreated culture medium, but no growth occurred in either, while a similar

sub-inoculation made from an inoculated control tube of the same age produced turbidity within 24 hours.

The coccus-like forms were produced consistently only in bouillon containing 4—7 % alcohol. This morphological change cannot be attributed to any action on the part of the alcohol upon the nutrient constituents of the medium that would render them less assimilable and it seems piobable that at these concentrations the bouillon remains practically unaltered as is shown by the fact that when growth at length does commence (as indicated by the first signs of turbidity) it proceeds approximately at the normal rate. Thus a culture with 5 % alcohol first showed a very slight turbidity on the seventh day after inoculation; at the end of the succeeding 24 hours the turbidity was quite pronounced and there was a little sediment, this being the condition assumed by a tube of normal bouillon 24 hours after inoculation. Microscopic examination of a drop of the turbid liquid of this culture showed the bacillus in the form of cocci even at this early stage of its development when food-stuffs must have been abundant. Two "poured plates" were prepared from this culture: one produced numerous minute colonies in which the individuals were again coccus-like, the other gave rise to five colonies each of which was examined and found to consist of the bacilliform motile rods. Assuming that no contamination had occurred it would appear that when the coccus-forms are sown thickly so that continued multiplication is arrested at a comparatively early stage that form is retained, while when they are fewer in number growth continues for a longer time and they revert to their normal shape and size. The experiment was repeated using another tube of bouillon containing 5 % alcohol with similar results; in 48 hours the plate in which the cocci had been sown thickly had very numerous minute colonies of coccus-forms, while the other had well-isolated colonies (though fairly numerous) up to 2·5 mm. in diameter and these consisted of typical rods.

Alcohol is well known to have dehydrating properties and it may be that its presence in the culture solution creates a condition of physiological desiccation which at first inhibits growth altogether. The isodiametric forms induced by the alcohol are probably more resistant to its action than the normal rods, since they offer a smaller surface to its dehydrating influence.

The shape and size of these coccus-like forms may conveniently be demonstrated by preparing a smear on a cover-glass in the usual way with a drop of the bouillon containing them; after drying and fixing

the film by passing through a flame it is stained with Heidenhain's haematoxylin. The differentiation with the mordant after staining is continued until the organic compounds of the bouillon are decolourized leaving only the bacteria stained (Fig. 6).

It would seem that the abnormal forms here described have not come under the observation of previous workers on soft-rot organisms. Harrison says (12) of *Bacillus oleraceae*, "Involution forms are commonly found. Thus the bacteria may be ovoid, or long and bent, occasionally club-shaped individuals are seen"; but the extreme forms apparently he did not observe. Jones writes (16) of *B. carotovorus*, "Pleomorphismus ist selten beobachtet worden. In verschiedenen, zwei Monate alten Kulturen (Röhren mit gedampften Möhren) hatten viele Stäbchen ovale, lichtbrechende Stellen in Innern, die stark an Sporen erinnerten,... sie für Vakuolen gehalten."

Erwin F. Smith in his monograph (36) figures[1] long filaments of *Bacterium campestre* from an old culture (about 5 weeks old) on 23 % grape sugar agar; he also finds[2] that old cultures of *Bacterium hyacinthi* "on media rich in sugar...often show many long slender chains and also filaments 50 to 150 μ long in which no septa are visible."

Miss Doidge shows (7) that *Bacillus mangiferae* in beef broth containing 7 to 8·75 % NaCl "grows out into long threads which are very variable in length and thickness"; some of these threads were swollen irregularly and apparently resemble those forms described above as occurring in the synthetic medium with 6 % alcohol.

IV. Cultural Observations.

Growth in Media containing Sugar. The bacillus was cultivated in Durham's tubes and in fermentation tubes in media containing peptone (Witte's) and one of the sugars dextrose, lactose, and saccharose prepared with litmus solution. Several batches of the media have been prepared and inoculated with the following general result. The media invariably developed acidity as indicated by a reddening of the contained litmus. 18 hours after inoculation (incubated at 28°—29° C.) the dextrose solution assumed a distinct red tint which approximated to that finally attained by all three; in the saccharose solution the reddening was evident but rather less intense, while with lactose there was but slight change from the original neutral tint. The reddening

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[1] *Loc. cit.*, Vol I, Fig. 19.   [2] *Loc. cit*, Vol. II, pp. 344–5 and Fig. 141.

however gradually became more definite and in 36 hours after inoculation all three media were of a bright red colour which was maintained to the end of the experiment. The sequence of colour changes appears to indicate that the bacillus is at first less vigorous in the presence of lactose than when dextrose or saccharose is the sugar available and this is borne out by the fact that in the earlier stages (up to 24 hours) the dextrose and saccharose media become turbid at a more rapid rate than that of lactose. It is associated too with a corresponding variation in gas production.

Bubbles of gas appear sooner or later in each of the three solutions after inoculation. Such has invariably been the case except in one experiment where gas failed to appear in two fermentation tubes containing the dextrose solution. With this exception bubbles always began to accumulate at the upper end of the tube (*i.e.* the inner tube in the case of a Durham's tube or the closed arm of a fermentation tube) usually in 20—24 hours after inoculation and are to be observed first in either dextrose or saccharose; dextrose seems to favour the early production of gas bubbles, though their appearance in saccharose solution follows shortly afterwards and may even precede that in dextrose. Gas does not appear in the tubes containing lactose until a few hours later but its production then proceeds at a relatively increased rate and continues for a longer period; consequently the final volume of gas accumulated in the tube is always in excess of that contained in the tubes of dextrose or saccharose.

Harding and Morse (11) found that various bacilli of the group of soft-rot organisms gave very variable results when grown in bouillon containing one or other of the three sugars above mentioned, even the same strain does not always give even approximately similar results in different experiments, this may be partly due to variation (in chemical composition and concentration) in the bouillon itself and it would be preferable to employ standard synthetic media for the sugar fermentation tests. The results recorded above were obtained by growing the bacillus in a medium containing peptone (a complex organic substance) in addition to the sugar, and those by Harding and Morse from sugar media prepared with sugar-free bouillon as a basis.

Repeated experiments with synthetic media containing saccharose as the only source of carbon have yielded no free gas whatever, although turbidity and sedimentation with distinct acidity were produced within 24 hours of inoculation. Thus in the absence of other organic compounds the final gaseous decomposition products of saccharose

may be evolved in quantity so small that they are dissolved in the medium as produced. The results suggest that the organic compounds of the peptone and bouillon may contribute to the production of gas when sugar is present.

Media containing Pectin. The experiments with synthetic media containing saccharose showed that the organism is capable of maintaining existence and propagating itself in media containing a sugar as the only source of carbon. The plants susceptible to attack by the bacillus are however not necessarily rich in sugar and the probability is that in nature (*i.e.* when living in plant tissues) the requisite carbon is derived principally from the pectic compounds of the cell-wall and particularly the middle lamella; the bacillus is unable to ferment pure cellulose as shown in an experiment in which it was grown in bouillon containing strips of Swedish filter-paper without any change occurring in the latter, and its action on starch is also practically negligible.

Artificially prepared culture media containing pectin instead of sugar have proved favourable for the growth of the organism. In the earlier experiments of this series a solution of pectin was prepared from ripe fruit by the method adopted by Buller (5). Later the gelatinous mass obtained from fruit-juice by its precipitation with alcohol was dehydrated by absolute alcohol and the latter finally driven off by gentle heat in a drying oven at about 40ᶜ C.: in this way the substance was obtained in a dry state and solutions of known strength could be prepared.

When pectin was used instead of sugar in combination with peptone and litmus a dense precipitate was produced on sterilizing. To obviate this, solutions of peptone and pectin were sterilized separately and then mixed.

A.   Peptone   2 gm.
     Distilled Water   50 c.c.
     Steamed for half an hour to dissolve the peptone.

B.   Pectin (dry)   1 gm.
     Distilled Water   45 c.c.
     Steamed until the pectin was dissolved then 5 c.c. litmus solution
     added.

The solutions A and B were sterilized separately, then mixed and put into sterile fermentation tubes, this process being carried out over a steaming sterilizer to avoid contamination. Of five tubes so prepared and incubated four were inoculated in succession at intervals of 24 hours,

the fifth being kept as control and for comparison. 24 hours after inoculation each tube showed a slight reddening of the litmus (compared with the uninoculated) and a dense sediment was beginning to accumulate in the basal part of the tubes. After another period of 24 hours the red colour was more intense and there was a copious sediment while the liquid itself was more transparent[1]. Within six days the liquid above the sediment was clear and almost colourless. Later the contents of the fermentation tubes were filtered; the filtrate was clear and colourless, the sediment remaining on the filter. That portion of the original culture medium still remaining in the uninoculated tube passed through the filter unchanged, leaving behind no sediment.

A solution in which pectin was the only source of carbon was also used and prepared as follows:—

Ammonium nitrate	1·0	gm.
Magnesium sulphate	0·04	,,
Dipotassium phosphate	0·25	,,
Sodium chloride	0·25	,,
Pectin (from apples)	1·0	,,
Distilled water	100·0	,,

This, when sterilized, was found to give a distinct acid reaction and the organism made but slight development in it. On neutralizing the medium with a solution of NaOH inoculated tubes became turbid in 24 hours and a distinct sediment was present after four days; on testing with litmus the tubes gave a pronounced acid reaction not shown by controls.

Pectin therefore may be substituted for sugar in the preparation of culture media for the celery rot organism and probably also for other soft-rot bacilli; since pectic compounds are readily attacked by these forms and as this ability to render soluble the middle lamellae of the tissues is the primary cause of the damage done to succulent organs of many garden and field crops, such culture media might yield interesting results relative to the mode of parasitism of soft-rot bacteria.

Uschinsky's Solution. Growth in this medium is vigorous, turbidity being evident within 24 hours of inoculation; flocculi then appear together with some sediment during the second day. After a few more days the medium becomes almost clear again and there is a bulky sediment which eventually forms a dense glairy mass at the bottom of

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[1] Owing to the concentration of the pectin the medium before inoculation is only semi-transparent.

the tube. No gas was ever evolved in Durham's tubes containing this solution.

For some time it was considered that no acidity was developed in this medium, repeated tests with litmus solution failing to indicate what could be considered as evidence that an acid was produced. The diffusion of the sediment through the liquid when the test was applied rendered a comparison with control tubes unsatisfactory, and passing the liquid through filter-paper failed to remove the suspended particles. More recently however another method was adopted. The tubes (of cultures a fortnight old) were placed in a water-bath which was then heated to boiling point when the tubes were removed and the contents filtered separately into other tubes through Swedish filter-paper. Six drops of neutral litmus solution were then added to the clear filtrate of each tube and also to the control tubes. An acid reaction, though faint, could then be detected in the inoculated tubes particularly if they were held, together with the control tubes, in such a way that the observer looked down the open ends of the tubes upon a white surface.

Since the celery-rot bacillus produces an acid from a glycerine containing medium (as Uschinsky's Solution), it should receive the number ·0000002 instead of ·0000003 as previously recorded (46) by the present writer and its full group number becomes 221·1113522.

This number approaches so nearly that of *Bacillus carotovorus* Jones (221·1113022) as determined by Harding and Morse (11) that it was thought desirable to obtain this organism for comparison I was able to obtain a culture of this *Bacillus* from Professor V. H. Blackman who kindly prepared and forwarded a sub-culture from a tube received by him from Dr Erwin F. Smith, Plant Pathologist to the Department of Agriculture in the United States. The two points on which a comparison was particularly required were (1) behaviour in Uschinsky's solution, and (2) chromogenesis.

From the tube of *B. carotovorus* (culture only two days old) two successive sub-cultures were made within five days on celery extract agar. In contrast to the celery organism its growth on this medium was comparatively slow and after 24 hours it was practically confined to the line of streak instead of spreading laterally as is the case with the celery bacillus. The difference in rate of growth of the two strains on celery agar may be a varietal character; on the other hand it is possible that the reaction of the carrot strain to vegetable decoctions had become modified from continued growth on a meat extract medium.

A difference was also to be observed when the two strains were

grown in Uschinsky's solution. Four tubes of the solution were inoculated from celery agar cultures, 48 hours old, with each of the two strains with the following result:—

	48 hours	7 days
B apiovorus (4 tubes)	Turbid, numerous flocculi, distinct sediment.	Liquid almost clear, sediment bulky.
B. carotovorus (4 tubes)	Growth feebler than in above, less turbid, flocculi less numerous, trace of sediment.	Turbid, sediment thin.

The general appearance of the tubes was maintained for five days longer (when they were tested for acidity), those of *B. apiovorus* remaining almost clear but with a dense bulky sediment, while those of *B. carotovorus* were still turbid, the sediment forming a comparatively thin layer; all gave a rather feeble acid reaction which however was unmistakable when the tubes were examined as described above.

V. CHROMOGENESIS.

In my previous papers the celery bacillus was described as a yellow organism as indicated by its colour when growing on sterilized potato in Roux's tubes, while *Bacillus carotovorus* is included by Harding and Morse (11) among the non-chromogenic bacteria, and in their résumé of its cultural features they write "Chromogenesis, *white on all media*."

It was found that when the two were grown on the same medium no difference in colour could be detected. Streak cultures of each have usually a yellowish tinge and this is also often seen in the sediments of cultures in liquid media. The colour is however more pronounced when they are grown on solid media such as sterilized potato or the Soyka Milk-Rice medium[1], the latter being particularly favourable for investigation on chromogenesis. Cultures of each were started at the same time on sterilized potato in Roux's tubes and on milk-rice and kept at the room temperature (18° C.).

On potato at the end of 24 hours the colour of the growth in both strains was practically that of the potato itself, the only difference being that the surface of the potato was more or less glistening with moisture while the bacterial mass had a matt surface. On the following day yellowing was noticeable and on the fourth day the colour of the culture was approximately "Cream Buff" of Ridgway's

[1] See Eyre's *Bacteriological Technique*, p 189, where the method of preparing this medium is given.

Scheme (34). After ten days the colour approached "Honey Yellow," the surface being by that time glistening and smooth.

The milk-rice was prepared in small petri dishes and in order to obtain exact comparison each plate was inoculated with both organisms, the points of inoculation being about 3 cm. apart; the growth on this medium was so localized that there was no danger of inter-contamination. On these plates the colour was identical with that on potato except on one plate (this probably contained a little less moisture than the rest) where it was "Naples Yellow."

Although *B. carotovorus* has been described as a white organism it appears to have been recognized that the colour was not a pure white. Harding and Morse refer to its "creamy growth" and Jones describes a "cream-white layer." Harrison in his description of *B. oleraceae*, which Harding and Morse find is "clearly identical with *Bacillus carotovorus*," says its growth on potato (Roux's method) is straw-coloured but that there were minor differences in various tests; "thus the growths would be dirty yellow, or honey yellow in colour."

The method usually adopted by the American phytopathologists when preparing potato cylinders as culture media is to place the half cylinder in an ordinary test tube containing a little water so that the basal portion of the potato is in the water itself; the whole cut surface remains more moist under these conditions than when a Roux's tube is used. By the latter method development of the organism is more localized and the colour more intense than when the former is employed. In Roux's tubes the yellow colour of the celery bacillus has always been clearly recognized on potato, and the Soyka milk-rice cultures not only confirmed those results but show that *Bacillus carotovorus* is also a yellow form; both must therefore be regarded as possessing yellow chromogens. This character in the numerical system of recording is represented by ·00005; the group number of *Bacillus carotovorus*. Jones, when modified in accordance with this, becomes 221·1113522 and the celery-rot organism will then be included under the same number.

The slight differences to be observed when the two are grown in Uschinsky's solution and on celery extract agar may be due to physiological modifications induced by the method of culture subsequent to isolation; in any case it would seem that the form obtained from celery and named by the writer *Bacillus apiovorus* may at most be only a variety of *Bacillus carotovorus* Jones.

VI. Effect of Desiccation.

Small circular cover-glasses were cleaned and placed in absolute alcohol. When required they were removed from the alcohol with flamed forceps, rapidly dried over a bunsen flame, then passed once or twice through the flame and placed in a sterile petri dish. A celery extract culture, two weeks old, was shaken to distribute the sediment throughout the liquid, then drops were transferred with a platinum loop to the cover-glasses on which they were spread and allowed to dry at the temperature of the laboratory, viz. 15° C. Two of the cover-glasses were taken up with flamed forceps when the smear was only about half dry and each was dropped into a tube of a culture solution. The rest were kept under careful observation and when the last visible trace of moisture had disappeared from any one smear the time was noted. Two were dropped into tubes of the culture solution when just dry, others at quarter hour, half hour and one hour respectively after first appearing dry. All the tubes were then incubated.

The result was as follows:—

		Condition of tubes after receiving the cover-glasses		
		24 hours	48 hours	5 days
(1)	Smear half dry	Both tubes very turbid	Turbid	Turbid
(2)	Smear just dry	Slight trace of turbidity in one tube, the other clear	Both very turbid	Turbid
(3)	¼ hour after drying	Both clear	Both turbid	Turbid
(4)	½ ,, ,, ,,	,, ,,	,, ,,	,,
(5)	1 ,, ,, ,,	,, ,,	Both clear	Both clear

When the film was not quite dry the result, as was to be expected, was as in an ordinary inoculation. Growth was retarded if the film was allowed to become dry even for a few moments and an hour's desiccation killed the organism.

In a similar experiment performed when the laboratory was at a higher temperature, viz. 18° C., desiccation for half an hour was sufficient to render the cover-glasses sterile.

A variation was also adopted by sterilizing and thoroughly drying test tubes which were incubated for a short time until they had acquired the temperature of the thermostat, i.e. 25° C., when smears were made near the bottom of each and allowed to dry at the same temperature. A few cubic centimetres of sterile celery extract were then added to each at periods varying from the time when they were just-dry to one hour afterwards. No growth occurred in any of the tubes, so that it

is evident that at 25° C. the organism is killed as soon as all visible trace of moisture has disappeared.

Jones (15) working with *B. carotovorus* found that in some cases several hours of desiccation were necessary to kill the organism even at a temperature of 28°—31° C. He used broth cultures and suggested that a surface film was formed which offered protection to the bacilli. On diluting the broth the organism proved much less resistant, two minutes' drying at a temperature of 22° C. sterilizing the cover-glasses. The latter result conforms more closely with the experiments recorded above and it is evident that celery extract on drying does not yield an effective protecting film.

SUMMARY.

1. A bacillus isolated from celery affected by soft-rot reproduced the disease when inoculated into celery plants.

2. Other vegetables commonly grown as garden crops are susceptible to attack by the same organism.

3. The bacillus is very sensitive to the action of antiseptic and germicidal reagents.

4. It is typically bacilliform but under certain conditions may be almost isodiametric and coccus-like, or it may develop into very long filaments.

5. It is capable of growing in a synthetic medium containing a sugar or pectin as the sole carbon compound.

6. Its group number is 221·1113522 and since *B. carotovorus* Jones proves to be a yellow organism the latter must also be included under that number. The two organisms appear to differ slightly on minor points, *e.g.* vigour of growth in Uschinsky's solution and on celery extract agar.

7. Like *B. carotovorus* the celery-rot organism is very sensitive to desiccation.

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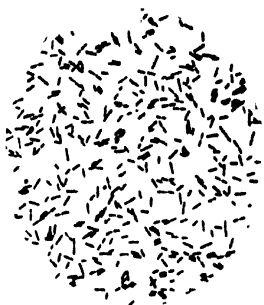
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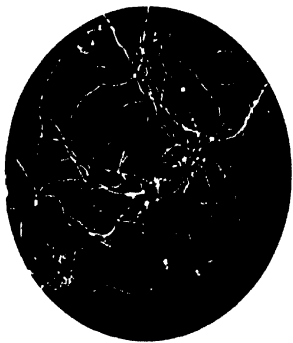
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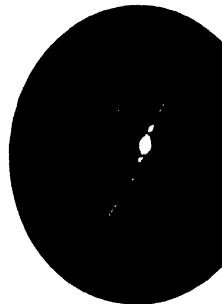
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EXPLANATION OF PLATES I AND II.

Fig 1   Three celery plants, the middle one control, the other two were each in oculated on 4 petioles   Result after seven days—all inoculated leaves, except one, rotten to base

Fig. 2.   Potato haulms five days after four had been inoculated, one of these has collapsed.

Fig 3   Two young turnip plants kept under the same bell jar   result 13 days after the one on the left was inoculated on five leaves   control plant on the right

Fig 4   The Celery Rot *Bacillus* from a young agar culture, stain Heidenhain's Haematoxylin   × 600

Fig 5   The bacillus stained to show flagella   × 600.

Fig 6   Coccus and diplococcus like forms of the bacillus from a bouillon culture containing 5 % alcohol   × 600

Fig 7   Filamentous forms of the bacillus from a bouillon culture containing 0 1 % benzoic acid.   × 600

Fig. 8   Filaments from a three week's old culture in a synthetic medium containing 0 1 % ' lysol substitute "   × 600

Fig. 9   A "nodulose" filament from a culture in a synthetic medium containing 6 % alcohol   × 600

Fig. 10   Microtome section of inoculated celery petiole showing the partial separation of the cells under the action of the organism   × 250

Fig 11   Microtome section of inoculated celery petiole showing portion of a cell-wall in surface view with bacilli *in situ*   × 500

BLACK CURRANT EELWORM.

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A DISEASE, which is due to a hitherto unrecorded attack of eelworm, has been found causing a considerable amount of damage to black currant plantations in the vicinity of Cambridge.

There is evidence that the parasite has been established for some years, but owing to its close association with the black currant mite, *Eriophyes ribis*, it has hitherto escaped attention.

The symptoms produced by the two diseases are similar in many respects and the economic importance of the nematode was therefore not suspected until microscopic inspection constantly revealed the presence of the worm living in unison with the acarid parasite.

It is evident, now that the life history of the worm has been dissociated from that of the mite, that it is responsible for at least an equal share of the damage attributed to that parasite.

In order that the essential differences between the two pests may be recognised, the following brief outline of similar and contrasting features in the life history of each is given.

Points of similarity. Both parasites are true ecto-parasites, and both are concerned with the ultimate destruction of the bud. Both are therefore controlled by the same factors, *i.e.* the seasonal routine of the tree in the production of buds and their subsequent development into shoots.

Thus there is a period in the life history of both pests when a nomadic existence is led between the young folded leaves, while the buds are in a rudimentary condition following which is the longer period spent within the developing and mature buds.

Both gain entrance to the buds between the scale leaves from which they pass on to the true leaves.

In both cases, reproduction, although apparently stimulated at certain seasons and checked at others, continues throughout the year.

Superficially the injury to their host by both mite and worm is identical, that is, the destruction of the buds and the diversion of the growth resulting in the loss of vitality to the tree are constant in both diseases. There is however a difference in the degree in which these symptoms occur.

Points of dissimilarity. No abnormal growth of the bud occurs as the result of an eelworm attack. Big buds, though worms may be present in them, are due to the action of mites alone.

A distinction is seen in the appearance of the bud leaves attacked by the two pests. Those recently invaded by worm shew, where colonies have become established, isolated discoloured areas which become confluent as the disease progresses. Accompanying this discoloration is a conspicuous state of moisture in which the diseased portions assume a transparent appearance. Such buds are incapable of further development.

These symptoms maintain in the buds successively attacked throughout the year.

Such discoloration does not occur throughout the year in the mite attacked buds. A certain darkening of the tissues may occur in isolated cases during the autumn months, following which discoloration is general among doomed buds. There is, however, an opaque appearance of the dying tissues, and the state of moisture is entirely absent.

An essential difference occurs between the migrations of the two pests. The worms, by reason of their comparatively rapid disintegrating action on the tissues of the bud, are forced to migrate at frequent intervals as the buds die throughout the year. The mites however migrate definitely in the spring of the year, and with the exception of quite minor migrations (which apparently occur among individuals throughout the summer and autumn months) stay within the buds they enter, until the following spring.

The action of the mites on the leaf tissue is not one of disintegration, but is essentially stimulating. It causes the bud leaves to increase perceptibly and the whole bud to enlarge.

Such briefly are the main points of distinction between the two pests, and it now remains to consider the biology of the nematode in detail.

Life history. It has been seen that the worms enter the buds, with the brief exception alluded to, at all seasons of the year. That

the black currant bud should present no difficulty in the matter of invasion is not surprising, seeing that this parasite measures at its greatest breadth, one half that of the acarid whose facility for entering the buds is notorious.

The worms are gregarious in their habits and enter the buds in colonies composed of individuals varying in number from a few dozen to many hundreds. When within the buds their number increases by reproduction, and conceivably by the arrival of additional migrants.

Reproduction continues throughout the year. There appears however to be a preponderance of females over males in the spring of the year, and eggs and larvae are extremely abundant during that period.

Experiments undertaken with the desire to discover details of the larval life of the worm, gave in all instances negative results. It was found that to keep the young worms on dissected bud leaves for any length of time in a sufficiently moist atmosphere, was an impossibility, as the worms invariably fell a prey to bacteria and the host to fungoid diseases. The method of infecting sterile host plants with eggs was also tried, but the delicate bodies were either damaged by the transference, or from other causes failed to develop in requisite numbers for the accurate description of the successive larval stages. Several artificial media were tried, but the worms failed to develop in them. The experiment had therefore to be abandoned as impracticable.

The habits of the worms within the bud are lethargic, their movements being so slight that the dissection of an attacked bud causes the worms which adhere to either side of the separated leaves to shew a slight recoiling movement only. They may thus, when a low powered dissecting microscope is used, be readily overlooked among the hairs that fringe and cover the leaves of the bud, being approximately of the same length, breadth, and colour, as these structures.

A characteristic grouping of worms takes place when the colonies have become strong within the buds. Such grouping is at once interesting and enlightening, in that it presages migration.

Thus on dissecting a bud in which the worms are numerous, it is seen that between the leaves, and especially at their extreme bases, bodies are present which closely resemble fragments of cotton wool, or the strands of some densely interwoven hyaline fungoid mycelium. Such bodies, which can be seen without a lens, and lifted intact on a dissecting needle, are, when examined microscopically, seen to be composed of worms in the aggregate, densely interwoven, and longitudinally extended. Many hundred worms are found in these closely

compacted masses, and all stages from egg to adult are present in them.

If such colonies are observed in dissected buds kept in a state of normal moisture for a few days, they shew no apparent diminution or increase in numbers, nor do they appear to alter their position in the bud. When, however, they are transferred to water they separate rapidly, and assume the quick wriggling motion characteristic of the group Anguillulidae when immersed in that medium. If, on the other hand, such infected buds after dissection are allowed to dry in the laboratory the worms will be found on examination to have grouped themselves in similar colonies, and to have taken up their position in the scale and outer leaves of the dried buds. They are found in a desiccated and quiescent condition, and the individuals comprising the groups are—instead of being intertwined and straightly extended— intercoiled, and form in many cases circles and figures of eight of the most perfect symmetry, notwithstanding the fact that they are composed of hundreds of individuals. The habit of the nematodes in thus forming colonies and the response of such bodies to the conditions of moisture and drought, are the principal factors which control the migration of the worms.

It has been observed in the field, and confirmed by experiment, that bud to bud migration, which recurs at frequent intervals throughout the year, depends entirely on the presence of the requisite degree of moisture in the form of rain, mists, or heavy dews, for its successful achievement. This being present, the worms can leave the dead buds, which in their contracted condition allow the water free entrance, and swimming rapidly in the film of moisture which envelops the intervening stem, can either ascend to the unattacked buds above or descend to those below. Arrived thus far, there is no difficulty in gaining an entrance between the unresisting scale leaves of the bud about to be attacked.

If, however, drought ensues when migration from a dead bud has become imperative, the worms undergo a period of enforced quiescence and desiccation in the scale leaves till a moist medium once more prevails. If such moisture be forthcoming in the form of light rain, heavy mists, or dews, the worms, after a certain period of immersion, gradually resume their motility, and behave as in the foregoing case. Should, however, torrential or heavy rain fall the desiccated migrants floating on the descending water are rapidly carried to the ground unless some intervening obstacle present itself to detain them. When

arrived there they may, on resuming their activity, reascend the tree, or proceed to a neighbouring bush, if moist conditions prevail for sufficient time to enable them to travel there.

That wind may act as a distributing factor of wider range when the worms are in a quiescent condition is highly probable but difficult of observation. The scale leaves of the dead buds become extremely brittle in dry weather, the slightest movement being sufficient to detach them from the main body of the bud. Such leaves with the attached colonies of worms adhering to them, would in this manner be carried by the wind to some considerable distance.

Such briefly are the habits of the worms and the conditions controlling them at the migratory period. The various stages will, however, be reconsidered later and in greater detail when experimental work dealing with migration is described.

The life history of the nematodes within the bud has now been outlined, and the effect of the parasites on the vegetative parts of these structures follows.

From the examination of numerous newly attacked buds it is seen that the worms generally adopt one of two methods of attack. They either, (i) group themselves at the bases of the scale leaves, or (ii) distribute themselves throughout the entire bud.

In the first case the scale leaves are the object of attack. These function as protective organs and are therefore more resistant and less succulent than the true leaves There is however at their extreme bases a ring, limited in area, of very succulent tissue Here the worms collect, and by the constant perforation of the tissue in this region, cause the vessels which are of vital importance to the life of the leaf to collapse. The decay of the whole structure, thus cut off from its source of supply, rapidly ensues, and a condition of decay, in which the worms live and multiply, is set up.

The latter case, in which distribution throughout the bud occurs, is one of peculiar interest, in that it throws light on the habits of the worms in relation to the bud mechanism. It has been suggested in a previous paper[1] that the oil-glands of the black currant bud are to a certain extent responsible for the attack of *Eriophyes ribis*. The following observation tends to confirm that theory.

The glands in question are epidermal outgrowths, and cover at close intervals the outer surfaces of the scale and true leaves of the buds of *Ribes nigrum*. They are considerably raised above the surfaces

[1] *Journal of Agricultural Science*, Vol. VI, Part II, p. 121.

of the leaves which bear them, and it is therefore these outgrowths which are in actual contact with the inner smooth surface of the enfolding leaves. The spaces thus formed between the glands, allow the worms to pass between them, and though the glands never appear to be attacked, the interstices are noticeably so.

Thus the dissected leaves of a diseased bud will, at an early stage of attack, shew a complete reproduction in sharply contrasting colours of the gland-bearing leaf on its opposing glandless leaf. The glands are represented by small bright green circular areas on the glandless leaf—proving that the worms are unable to attack the parts so protected—while the tissues between them are attacked and discoloured by the action of the worms.

Such evidence therefore leads one to suppose that were the glands absent, and the bud scales more closely appressed, neither worm nor mite could gain entrance between them.

At this stage of the disease the condition of moisture, associated with which is the transparent appearance of the injured tissues, begins to shew itself. Both symptoms are characteristic of eelworm attack, and are alike caused by the exudation of sap from the injured tissues. The transparent appearance of the attacked portions is invariably present in the initial stage of attack before decay has set in, but the amount of moisture apparently depends on the position of the attacked bud. Thus the terminal buds, always highly developed, shew by their excessive state of moisture under attack, that a higher concentration of sap is present in them than in the lateral buds, which occasionally exude but a negligeable quantity under the same conditions. It is interesting to note that this excess of moisture which is apparently a necessary medium for the nematode, is not one that will support the life of the other inhabitants of the attacked bud, for the mites and their chalcid parasites are often, when sought for microscopically, found to be entirely immersed and drowned in the sap exuded by the leaves.

The rapidity with which an attacked bud is reduced to a state of decay depends on two factors, (i) the time of year when the buds are attacked, and (ii) the strength of the colony invading them. Thus in the spring of the year when the buds are minute, the rate at which they are destroyed is alarming; later, however, when they are more developed and especially when they are stimulated into abnormal growth by the action of *Eriophyes ribis*, the decay is not so rapid. Laboratory experiments shewed that from five to six weeks élapsed from the date of inoculation to the death of the fully developed mite-

free bud. It is probable, however, that under orchard conditions, decay would take place more rapidly, for the number of worms with which the buds were inoculated in the laboratory, was necessarily small.

It is obvious that the continuous destruction of the buds influences the growth of the tree. Especially is this the case during the spring and early summer when new growth is being actively made. If, for example, a shoot known to be infected with worms is kept under observation in the spring, it is seen that the wood buds which have escaped attack will push into growth. These shoots, proceeding as they do from centres of infection, may grow a few inches and then suddenly wilt and die, owing to the worms piercing the delicate tissues of the young stem and destroying the developing leaves. The concentration of the sap in the unattacked buds below such an abortive shoot usually causes several of the nearest unattacked buds on the old wood to break into shoots   These may, if growth is being rapidly made, attain to the length of from six inches to a foot. Upon examination of the minute buds they bear it will be found that few have escaped attack. The following figures were taken from such shoots in the first week in April:

Shoot of 30 buds shewed 28 killed with 2 expanding normally.

,,	24	,,	,,	19	,,	,, 4	,,	,,
,,	21	,,	,,	21	,,	,, 0	,,	,.
,,	16	,,	,,	14	,,	,, 2	,,	,,
,,	10	,,	,,	9	,,	,. 1	,,	.,

The terminal buds on such infected shoots are invariably killed, as are many of those immediately below. If all the buds at the apex of those new shoots are successively attacked (and such is a frequent occurrence) the shoot dies back to the nearest unattacked bud. The new basal wood which considerably influences the yield of fruit in black currants, is similarly attacked and destroyed.

Repeated efforts are thus being made by the tree during the growing season to produce new wood with the result that an irregular growth of twigs is produced about a diseased area. During the resting season no alteration takes place in the growth of the tree, but the worms continue to destroy the buds   In the spring long lines of unexpanding buds, interspersed with a few which are developing normally, shew the effect of the activity of the worms during the months when the trees remain dormant   Such a condition is shewn in the accompanying text figure.

The irregularity in the growth of the wood is most characteristic of an eelworm attack, and the presence of dead or partially dead shoots, with the bark shrivelled, the end tapering and thread-like, together with a cluster of weak shoots, are typical signs of the presence of the black currant eelworm.

The foregoing observations are the outcome of work carried out conjointly in the field and laboratory. The latter was found to be indispensable in working out the life history of this nematode. Especially was this the case in dealing with the difficult period of

migration, when the comparative invisibility of the worm when immersed in water, rendered the task of following its movements in the field impossible.

The following experimental work was undertaken in this connection and it deals, (i) with migration, which includes some observations on the desiccation of the worm, and (ii), with the inoculation of various plants with the object of discovering, (a) whether trees found normally associated with the black currant were likewise host plants for the parasite, and (b) whether the worm could be identified with other members of the group Anguillulidae causing specific diseases among various plants.

In dealing with migration, the necessity was evident for discovering the degree of susceptibility of the worm to the atmospheric conditions which maintain in the open.

An experiment having this object in view was carried out in the laboratory roof greenhouse, the isolated position of which rendered infection from any source remote. Black currant seedlings raised for the purpose under sterile conditions were used as host plants and the experiment was carried out during the winter months, while the buds were in a resting condition. The greenhouse was maintained at an equable temperature, and the water used for spraying and for ordinary watering, was distilled. Both soil and pots used throughout the experiment were sterilized. Inoculation was effected either by placing buds detached from infected material on the soil round the seedlings, or by directly introducing colonies of worms between the scale leaves of the buds. The experiment was continued over a period of six weeks from the date of inoculation, after which the buds which averaged 150 in number exclusive of controls in each section, were microscopically examined.

On material thus prepared, it was hoped, by the strongly contrasting conditions of drought and moisture, to gain information on the following points :-

(i) Whether migration could take place under conditions in which moisture is entirely withheld from the vegetative parts

(ii) Whether at the other extreme it can be successfully carried out if the vegetative parts are subjected to an excess of moisture

(iii) Whether an occasional vigorous application of moisture to resemble heavy rain influences migration.

(iv) And whether a similar light application resembling light rain and mists yields identical results.

SECTION I.

Whether migration can take place under conditions in which moisture is entirely withheld from the vegetative parts

The aërial portions of the seedlings used for this experiment were kept under conditions of drought, the buds and stems receiving no moisture in any form. Watering of the soil was however performed when necessary to keep the plants alive, and to ensure the activity of the worms with which the plants were inoculated.

It became necessary before this experiment was started to remove the basal buds immediately above the soil surface, for these became

covered with a film of water in the ordinary course of watering and the success of the experiment depended upon keeping all of the buds dry. It must be noted here that it was possible to almost eliminate atmospheric condensation in the greenhouse in which the plants were kept. Inoculation in this experiment was effected by placing diseased buds in a heap round the stems of the seedlings.

After a six weeks' period microscopic examination of the seedlings thus treated shewed that no infection by worm of the aërial buds of the seedlings had taken place. The basal buds, however, which are produced below the surface of the soil and which are often as well developed as the terminal buds of the shoots, were in all instances badly infected with worm and in an advanced stage of decay. The stems were dissected and examined microscopically as were the roots. They shewed however no sign of the presence of worm, neither was there any discoloration of the tissue such as would occur had the worms lived there parasitically.

It will be convenient as each section of the experiment is dealt with to sum up the points that appear to be established with regard to migration, and to follow up suggested lines as they occur.

The foregoing experiments appear to establish the following points:

(i) That the worms do not invade the buds if the stems of the plants are kept under dry conditions.

(ii) That they can, on the other hand, penetrate the soil and infect the basal buds beneath the surface under the condition of moisture supplied by the ordinary course of watering.

(iii) That they do not enter the roots of the plants and gain access to the buds by the internal tissues of the stems.

Ectoparasitic habits are thus suggested.

SECTION II.

In this section an endeavour was made to discover whether migration could be carried out if the vegetative parts were subjected to an excess of moisture.

For this purpose it was proposed to keep the experimental plants under strongly contrasting conditions to those of the foregoing section. The seedlings were therefore embedded to the rim of the pots in sand which was kept moist throughout the experiment and over the plants thus treated bell-jars, having their rims tightly pressed into the sand, were placed. In addition to this treatment the plants were sprayed night and morning with a fine spray producer. Inoculation was effected

as in the previous section by placing the diseased buds in close proximity to the stems of the seedlings. The plants and worms were in this manner kept throughout the experiment under the influence of a sufficient amount of moisture to maintain a continuous film of water over the buds and stems.

At the termination of the six weeks' period it was found that of the 150 buds kept under these conditions four only remained unattacked. The attacked buds shewed the typical symptoms of discoloration and decay and the worms in them were plentiful and reproducing freely. Especially were the terminal and apical buds and those beneath the soil surface in an advanced stage of decay and the number of worms contained in them was phenomenal. Examination of the stems and roots shewed that the worms were absent from these parts. The control plants in this section were in all cases found free from worm, but a certain amount of discoloration was present in many buds, which condition was however anticipated and accounted for by the unnatural state of moisture and airlessness in which they had been kept. Many of these buds had, under the influence of warmth and of moisture, pushed into growth and not a few were attacked by *Botrytis*.

Surplus control plants which were included in this experiment and not needed for dissection were grown on subsequently under normal conditions. These, with the exception of a few buds which failed to expand, developed normally, thus proving that the abnormal conditions under which the experiment had been conducted had not seriously influenced the results obtained

From this section of the experiment it may be concluded that under the influence of moisture the worms can ascend from the soil to the buds above the soil surface and descend to those below it. The results also tend to confirm the supposition that the worms are ectoparasitic in their habits and travel externally by the stem from bud to bud, and not by the internal tissues.

A further experiment acting more or less as a check on the foregoing section was carried out in the following manner. Pots containing soil only were inoculated as in the previous cases with diseased buds heaped on the soil surface. For a period of six weeks moisture was entirely withheld from these pots, after which time they were watered freely. Cuttings from disease-free seedlings were then inserted in the infected soil of these pots—half of the pots and cuttings were then transferred to the moist conditions of the previous section and half were kept under the conditions of drought as in Section I. At the

termination of this period, microscopic examination shewed that infection of those cuttings kept under moist conditions had taken place in much the same proportion as those previously kept under the same conditions, and that those kept under dry conditions remained unaffected. The roots made freely by the cuttings under both dry and moist conditions and also the stems were found upon dissection to be free from worm.

From the united evidence of these experiments it may be said that migration from the soil to the buds occurs only when the medium of moisture is present which enables the worms to reach their destination. It also confirms the ectoparasitic habits of the worms, for it is reasonable to suppose, seeing that the worms and the roots started into activity at much the same time, that the latter, had the worms penetrated them when they were in a rudimentary condition, would at least have had their growth checked or entirely suspended. This experiment also opens up the question of the suspension of the life of the worm under conditions of drought, which phenomenon will be discussed later.

Having thus ascertained that migration and distribution of the worm occur under conditions of moisture, it became necessary to consider the effect on migrating worms of these conditions as they occur in the open.

The following sections are therefore an endeavour to produce such conditions artificially in the laboratory, an attempt which, although clumsy as such experiments must be, yet gave results which are probably somewhere near the truth. They deal with conditions of an extreme nature, i.e., the influence of heavy rain and of light rain or mist.

SECTION III.

An endeavour was made to ascertain:—(i) whether worms under the influence of heavy rain could ascend from the soil to the apical buds of the seedlings if infection were restricted to the soil, and (ii) whether, if infection were in like manner restricted to the apical buds, they could infect those buds situated on the stem below them.

The seedlings required for this experiment were therefore divided into two series, in one of which infection was brought about as in the previous experiments by soil inoculation and in the other by bud inoculation, in which case colonies of worms were inserted directly into the bud scales of the terminal and apical buds. Both series were kept under identical conditions and the effect of heavy drops of rain

imitated by a daily overhead watering with a can the rose of which had large holes.

At the termination of the experiment it was found, contrary to expectation, that the buds of both series with the exception of a few cases were unattacked by worm. There was some difficulty in understanding such results. It was thought probable however that two factors, working either separately or conjointly, produced this comparative immunity in the seedlings. These factors were (i) the condition of inactivity of the worm at the time of inoculation, and (ii) the effect of forceful descending water on worms in that state.

In dealing with the first factor it is necessary to explain the method employed for obtaining worms for bud inoculation purposes in this experiment. This consisted in allowing diseased buds taken from the orchard to become sufficiently dry to induce the worms to form colonies in the scale leaves. In this manner they were obtained in great numbers and were convenient to handle. Such worms would not be in a thoroughly desiccated condition, but they would, on the other hand, be incapable of becoming immediately active when immersed in water. Taking this into consideration it is evident that a strong force of water coming in contact with colonies of worms loosely inserted in the scale leaves might carry them down to the soil before they could become sufficiently active to invade the buds in their descent. Those buds which became infected were, it is supposed, invaded by nematodes which had remained in the scale leaves until their activity was regained or which had reascended the stem from the soil.

The condition of the worms which thus influenced this section of the experiment dealing with bud inoculation, could not in the same manner have influenced that in which infection was brought about through the soil. In this case the worms would be kept active throughout the experiment by the moisture always present.

It is only possible therefore to conjecture that the rapid downward trend of the drops of water was sufficiently forceful to prevent the worms from ascending the stems of the plants in any numbers. In this connection it is of interest to note the conduct of drops of water as they fall upon the leafless stems of the plants. They collect at the buds, entirely immerse them for a few seconds, run quickly down and round the stem to the succeeding lower buds until the soil is reached. There is little doubt that in this manner migrating worms resting within the scale leaves in a desiccated condition, when they float readily on the surface of the water, are carried to the soil.

On the other hand should such worms be in a motile instead of a quiescent condition, so rapid are their movements that they could utilize the short period during which the drop of rain immersed the bud to penetrate the scale leaves.

There is yet another condition which may have influenced the results obtained in this experiment. The atmospheric condition of the greenhouse in which the plants were kept was unnaturally dry—this had the effect of causing the stems of the seedlings to dry rapidly after they had been watered, thus restricting the period of activity on which the distribution of the worm depends. Under normal conditions in the open the stems of the trees remain covered with a film of water for some considerable time after rain has fallen, especially in the case of moss-covered trees. Such a statement applies to normal rainy weather and not to heavy showers followed by brilliant sunshine.

SECTION IV.

To proceed to the latter part of the experiment which deals with the effect of light rain and heavy mists on migrating bodies of worms.

This experiment resembles the foregoing in that the plants were divided into two series and inoculated, (i) by means of the soil, and (ii) by direct bud inoculation; but differed in that they were sprayed twice daily with a spray producer. At the termination of the experimental period it was found upon examination that approximately one-half of the buds of the seedlings thus treated had become infected with worm. These results suggest therefore that under conditions of light rain and mists the worms are able to distribute themselves with greater ease than under conditions of heavy rain. This is probably the case under normal conditions in the orchard. Heavy mists are usually of lengthy duration and while they prevail the worms would regain their activity and proceed in the film of moisture, resulting from atmospheric condensation, to invade the surrounding buds.

The experimental work dealing with the migration, and the influence of atmospheric conditions on that period, has now been given. It is however important to realize that such work can at its best but roughly indicate the probable habits of the worms under normal conditions. The results of this experimental work are here given in tabulated form.

	Condition of vegetative parts	No. buds	Period of Expt	No. buds infected	No. buds beneath soil and 3 inches above infected	Other buds
I	Dry, soil inoculated	150	6 weeks	0	Removed	0
II	Excessive moisture, ditto	150	,,	141	130	11
	Representing heavy rain					
III	A. Soil inoculated	150	,,	40	37	3
	B Bud ,,	150	,,	36	23	13
	Representing light rain					
IV	A. Soil inoculated	150	,,	96	34	62
	B. Bud ,,	150	,,	114	24	90

Experiments dealing with the infection of probable host plants.

Having thus ascertained the lines along which migration proceeds, it became possible to deal with the infection of probable host plants.

The hosts chosen for the purpose were red currant and gooseberry, which bush fruits are normally grown in a mixed plantation. From a practical point of view, the question of interchange of hosts among surrounding trees is important, for if such interchange could be proved, it would necessitate remedial measures being extended to such hosts.

It must be noted here that in point of time this series of experiments was undertaken after the work on migration was complete. It was not until the month of January that the experiments were started and thus different conditions due to the advancing spring were unavoidably introduced.

The results obtained from such work are not entirely convincing. Some features however are fairly consistent throughout the series and they may therefore be of some value if they are not isolated from observations made at the same time in the field.

For the purposes of this experiment gooseberry and red currant seedlings were raised under the sterile conditions of the previous experiments and kept in the laboratory greenhouse under the conditions found to favour migration, (i) in excessive moisture under bell-jars, and (ii) uncovered and sprayed twice daily with a spray producer. The number of buds experimented on and the duration of the experiment were also those of the previous experiment, but inoculation was restricted to the soil.

At the termination of the experiment an examination of both species of Ribes shewed that infection had taken place in much the same proportion in both hosts. It was found that approximately 25 per cent. of infected buds occurred in those seedlings kept under the

comparatively normal conditions, and that a slightly higher percentage had occurred in those kept under the abnormally moist conditions.

It was here that the effect of the conditions due to advancing spring became evident, for under the influence of the artificial warmth and moisture of the greenhouse a certain proportion of the buds developed prematurely. Such development was however of interest in that a contrast between dormant and expanding buds and the relation of the worm to such expansion was evident. Thus it was found that with a few doubtful exceptions the worms had been able under these conditions to enter the developing buds, while those which remained dormant shewed the presence of worm in the scale leaves only.

The number of worms found in the infected buds was in all cases small, cast skins were however present in many from which living worms were absent. There was some evidence of decay in the leaf tissue on which living worms were found, but in no case was this sufficient to prevent the normal development of the bud. Such decay was absent from the controls kept under the normal conditions of moisture.

It is difficult to draw satisfactory conclusions from such results. They appear in the first place to suggest, seeing that the dormant buds remained unaffected except for the presence of the worm in the scale leaves, that the tightness of the true leaves of the buds prevented them from entering these parts and that when this condition was relieved by the expansion of the buds they were able to live there to some extent parasitically. On the other hand, repeated investigation of both hosts in orchards infected with the worm failed to shew their presence even in the scale leaves, nor were the typical symptoms of bud rot and irregular growth present among them.

The limited number of living worms found within the buds and the presence of cast skins only in so large a proportion of them suggest that the worms finding themselves on an uncongenial host had left such buds to seek new fields for attack. This suggestion is strengthened by the fact that although decay was present it was not comparable with the typical bud rot of black currants produced under similar experimental conditions.

It is therefore probable, notwithstanding the fact that experimentally the worms appeared to be living to some extent parasitically on the red currant and gooseberry, that these species of Ribes do not play an important part as hosts for the black currant worm.

The final section of this experiment deals with an endeavour to discover whether the worm causing bud rot in black currants could be identified with the members of the group **Anguillulidae** causing specific disease among plants.

Special importance was attached in this case to the experiments dealing with the infection of the strawberry as host. The necessity for such work arose owing to the suggestion by the eelworm specialists to whom the worm was sent for identification, that the nematode was probably identical with *Aphelenchus fragariae* which causes the disease known as "Cauliflower disease" on the strawberry.

The laboratory work in this connection was carried out under the same conditions and duration of time as that of the previous experiments, but the experimental work in the orchard was continued over a period of two years.

It was unfortunate that it was not possible to obtain sterile seedlings for these experiments   The nursery from which the plants were obtained however was apparently free from the particular disease. The so-called Cauliflower disease is a particularly obvious one and if present it could hardly be overlooked among several houses of forcing strawberries. Moreover the disease is a peculiarly local one and has only been recorded from a few localities in England.

As a precaution however, the plants to be used for the experiment were kept under running water for several hours and repotted in sterile pots and soil.

The variety used was Royal Sovereign and one dozen well-grown fruiting plants were chosen for each experiment. These plants were kept as previously described under the conditions found to favour migration, and soil inoculation was employed throughout.

The results obtained from the plants thus treated were curiously similar to those obtained in the previous experiment. Worms were found to have established themselves in much the same proportion between the young folded leaflets, especially in those plants kept under conditions of excessive moisture. Cast skins only were likewise present in many of the leaves. The hard white buds of the strawberry, the leaves of which are closely appressed, were however in no case invaded. Discoloured areas were present on those portions of the leaves on which living worms were found and such evidence of decay was absent in the control plants.

None of the characteristic symptoms of Cauliflower disease appeared however during the experiment, and plants kept for a period of two

months following inoculation remained normal in growth and flower production, and were setting fruit freely at the end of that time. Neither did the roots and stems shew signs of the presence of the worm when examined microscopically.

To all appearances the results of these laboratory experiments are identical with those of the previous section dealing with the inoculation of the red currant and gooseberry. The cast skins point to uncongenial surroundings and the limited number of living worms strengthens that view. Absence of the most conspicuous symptoms of distortion among the inflorescences, and the failure to find the worms within the roots and stems of this host after a lengthy experimental period, shew that it is at most highly improbable that this worm is related to the nematode causing "Cauliflower disease."

On the other hand the worms appear to have led a slightly parasitic existence on this host. It is important therefore that this experimental work should not be given undue prominence but that it should be if anything subordinated to those experiments carried on in the field under normal conditions.

Such experiments were carried out at Cottenham, Haslingfield and Grantchester, districts several miles distant one from another, where the black currant worm was found well established as a disease. Strawberry plants of the varieties Royal Sovereign, Laxton and President were taken to these districts and planted directly under the infected trees, and in all cases where infected basal wood was found, plants were placed immediately against them. Eighteen plants were allotted to each district and at frequent intervals diseased twigs were cut from the trees above and placed in direct contact with the strawberry plants which it was hoped by these methods to infect.

In the first season runners were made in great numbers by the plants. Some of these were severed from time to time from the parent plants and examined for the presence of worm, with negative results on each occasion. The parent plants were allowed to remain undisturbed, but signs of the disease were looked for during the season. They however flowered and fruited normally, although the fruits were small owing to the darkened condition under which they had matured.

The end of the first season shewed no sign of abnormal growth in the plants or any symptom that would lead one to suspect that the plants were suffering from the disease in question.

Identical results were obtained during the following season throughout which inoculation was continued as before. By the end of the

year a mat of stolons had grown round the parent plants. These were in all respects normal products and they proved to be free from worm when examined microscopically. The parent plants also were normal and were, when lifted and subjected to like treatment, found to be free from infection.

There is in this experiment carried out under orchard conditions confirmatory evidence that the worm causing bud rot in black currant is not identical with that causing Cauliflower disease in strawberry.

Finally, inoculation experiments on various seedlings including clover, oats, onions and wheat were also undertaken in the laboratory under those conditions found to favour distribution. Soil inoculation was employed throughout and the length of the inoculation period was that of previous experiments.

The results obtained were negative in so far that the typical symptoms exhibited by such plants when attacked by eelworm did not appear. There were however present in many of the seedlings localized discoloured areas in the leaf tissue on which small numbers of worms were found leading an ectoparasitic existence. They proved to be absent from the internal tissues of leaf, root, and stem, when examined microscopically.

Collectively the experimental data taken from the infection of probable host plants appear to shew that under abnormal conditions a weakly facultative ectoparasitic existence can be led.

This evidence is not however confirmed by observation and experimental work carried out under normal conditions in the field.

OBSERVATIONS ON THE DESICCATION OF THE WORM.

In the life history of the group Anguillulidae the desiccation of the living organism occurs at times when unfavourable conditions for distribution or for obtaining food persist. Should this state, in which active life is suspended and no food is partaken of, be maintained for months, or in some cases for years together, the worms can, on favourable conditions reasserting themselves, resume their active state. Thus in the case of *Tylenchus tritici*[1] the worm has been known to return to a motile existence after a period of 27 years of desiccation.

Seeing that from a practical point of view this habit is one of importance, the following observations were made on the habits of the black currant worm after certain periods of desiccation.

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[1] Bastian, H. C., "Monograph on the Anguillulidae," *Trans. Linn. Soc.* **xxv**, 1866 (read 1864), p. 86.

For the purposes of observation branches of black currant, the buds of which contained worms, were taken directly from the infected tree and hung up in the laboratory without being allowed to come into contact with the soil, from which medium infection from free-living species might occur. The branches were allowed to remain in a dry state for periods of 9 months, 6 months and 6 weeks respectively, at the end of which time microscopic examination of the worms thus treated took place. The worms upon which such enforced desiccation and starvation were thrust were found in all cases to have collected in colonies among the bud leaves in the manner described in the general life history. These colonies in their dry, quiescent condition were then transferred to a drop of water on a slide and the movements of the individuals thus placed in a medium in which activity could be resumed were observed under a microscope.

It has been previously shewn in the general life history that the worms in a state of quiescence adopt a coiled position within the colonies.

On being immersed in water a gradual uncoiling is seen to take place among them, until the body resumes its extended position and the body contents, which during desiccation recede from the epidermal cell wall, return to their normal position. These processes appear to occupy a length of time varying with the duration of the desiccation period. Thus the body contents of the worms which had remained in a state of quiescence for 9 months resumed their normal position against the epidermal cell wall after a period of 30 minutes' immersion. A further period of 30 minutes was however necessary before the worm became indistinguishable in appearance from specimens taken directly from the field. The extended position of the body was not general among the worms until a period of 5 hours following immersion had elapsed. Movements during this time were frequent among the nematodes, but they were of an irregular and spasmodic nature and were probably due to the expansion of the tissues and not to any voluntary muscular effort on the part of the worm. Following a period of from 20—30 hours of immersion a state of progressive activity could be distinguished among the worms under observation. This type of movement was exhibited by an extremely limited number and was characterized by incessant and ceaseless forward motion. Indeed, so swift and rapid were the movements of the active worms that it was impossible to determine their sex until they became weakened and their movements were consequently slower. This intense activity

continued apparently without cessation for a period of from 7—10 days, after which time death ensued. The number of worms kept under observation for this experiment was roughly 1400. and of these only 29 returned to the highly motile state described. 21 of these proved to be larval forms, 5 were females and the remainder were of undetermined sex. The limited number of worms surviving this experiment prevented any microscopic examination of them being undertaken, otherwise comparative results between these and the worms about to be described would be interesting. External examination of these worms after death for this purpose was not successful and the unexpectedly large amount of material used in this experiment left nothing for further experimental work on the subject.

The type of activity described above presented a sharp contrast to that exhibited by other worms in the colonies examined. This took place after the movements due to expansion of tissues had ceased and consisted of slow occasional movements during which the body was swayed slowly from side to side and drawn upward, while one half, usually the posterior, remained in a stationary position. Several minutes would elapse before such movements were repeated. The worms exhibiting this type of movement, which was shewn by approximately 30 per cent , never became actively motile, nor did they remain in a state of activity for so long a period as the worms previously described. The worms which shewed neither type of movement among the colonies consisted of (i) dead, and (ii) broken specimens, both of which occurred in much the same proportions.

An examination of the worms which had remained in a state of desiccation for the 6 months' period shewed that in this case the two types of movement were not distinguishable and that approximately 70 per cent. of the worms returned to an actively progressive state after a comparatively short immersion period of from 1—2 hours.

The results obtained from those which had undergone the same process for the 6 weeks' period shewed that fully 80 per cent of the worms returned to the normally active state after the short immersion period of from ½ to 1½ hours

It must be noted here that in many of the colonies examined there was strong evidence of parasitism to which the presence of numerous broken bodies of worm must be attributed. Predatory acarids are numerous on the black currant and it is possible that they are partly responsible for the considerable mortality which occurs among the worms in their quiescent condition.

The results of the foregoing experiments suggest the following points. That after a long period of desiccation the black currant worm loses to a great extent its power of resuming a motile existence. It is shewn on the other hand that it can survive such a state with comparatively slight mortality if the period of desiccation is reduced by a third.

If these results may be interpreted in the following manner there is reason to believe that a time limit exists past which the majority cannot live and it is suggested that the survivors differ at least in regard to vitality from those which succumbed to protracted drought.

It is also suggested that the worms which shewed a limited activity after a lengthy period of desiccation were dying out—they appeared totally unable to employ the usual method of progression and therefore they would be unable to use the means of distribution on which they rely. It is probable however even at this stage that they would be able, if brought into actual contact with their plant food from which they could directly draw nourishment, to become active members of the community.

The natural influences to which the worms are subjected under normal conditions must be considered. They are (i) the general climatic conditions, and (ii) the nature of the host upon which a parasitic existence is led. With reference to the former it may be definitely stated that at no season of the year is there a period of drought comparable to either of the two longest experimental periods. Again, when the perennial nature of the host plant is considered it will be seen that no occasion arises in which quiescence would be due to the failure of food supply, which condition renders quiescence imperative among worms whose hosts are of annual duration only. If therefore the black currant is the only host upon which the worm is genuinely parasitic, it is difficult to conceive a situation in which a period of prolonged desiccation would enter into the life of the organism.

Quiescence in this case would merely mean that distribution was temporarily suspended and would not be a question of tiding over a lean time with the probable chance of not coming into contact with food at the close of that time.

The heavy mortality under comparatively short periods of desiccation among a group of worms well known for their vitality under like conditions, would seem to be an additional proof that the black currant worm differs from other described parasitic species in this country.

In many biological details however the worm is comparable to *Tylenchus angustus* which nematode causes a disease of rice known as "Ufra" in India. On this host the entirely ectoparasitic habits of the worms which are so marked a feature in the life history of the black currant worm have also been established. Another point of interest is the failure of the rice worm to reach the growing point of the plant, which again results from the tightness of the final enveloping leaves of the bud.

In the biological work on the black currant eelworm an endeavour has been made to throw light on those periods which are of vital importance to the life of the organism and the relation of that organism to the natural conditions which surround it.

The morphology of the worm however presented difficulties which rendered comparison with closely allied species almost impossible. For these, hasty descriptions and inadequate drawings of the species previously described added to real difficulties in the matter of staining of the worm under consideration were responsible.

It was therefore with much gratitude that I accepted the help of Dr Assheton to whom I am indebted for the following description and some of the drawings which accompany it.

It is hoped that such biological and morphological work will at least form a basis for comparison or that it will prove of use should the worm appear on hosts other than that on which its parasitism has been established.

Morphological details.

The difficulty of determining the details of the internal anatomy with accuracy is great owing to the thickness of the chitinous cuticle which covers it and prevents the penetration of staining fluids. A combination of platinic chloride, acetic acid and osmic acid (Hermann's fluid) fixes and stains slightly the internal tissues and renders them more easily observable. If the worm is bisected on this fixation with Hermann's fluid and then left for 24 hours in Ranvier's Picro-carmine very fairly well stained pieces can be obtained.

External character. This nematode measures rather less than one millimetre in length—the largest specimen measured, which was a female, was ·92 mm. in length. The males as a rule are slightly smaller. In width the female is always slightly broader than the male, and when containing a fully formed egg it is markedly so, but it does not exceed ·04 mm. at its broadest point which is near to the middle

of the body. The ordinary female does not exceed ·03 mm. in the middle region. The body tapers gradually towards each end, and ends posteriorly in a point, but the front end is truncated and terminates in a slight expansion as shewn in Pl. III, fig. 3. This expansion seems to be due to thickened areas of the cuticle, probably specially developed in connection with the protracted muscles of the spear. The whole body is covered by a thick chitinous cuticle which is marked by well defined but very narrow circular striae which are due to some slight difference in the character of the substance of the cuticle and are not due to raised rings. There are also longitudinal lines of a rather coarse nature, but these are difficult to see and their nature was not determined.

The anterior ends of male and female are alike, but the posterior ends differ considerably. The mouth is at the extreme anterior end, the anus or cloaca is on the ventral surface a short distance in front of the extreme posterior end. There is, therefore, a short tail-piece. The reproduction duct is single in both male and female. In the male it opens upon the ventral surface into the cloaca just in front of the anus. The cloaca is bounded on its right and left sides by two folds of cuticle, a character here very slightly developed but which is a prominent feature in some species of Tylenchus. In the female the genital duct opens in the median line on the ventral surface some distance in front of the cloaca, but posterior to the middle of the body (Pl. III, fig. 2).

A conspicuous feature of the male is the presence of spicules developed in the cuticle of the cloaca and retracted ordinarily in a special pocket of the cloaca, which are described below. The tail of the male is shorter and blunter than that of the female and ends in a small papilla, the so-called ventral sucker (Pl. III, fig. 7). The tail part of the male is always flexed ventrally, whilst in the female it is usually straight. The female's tail is longer and more tapered, but ends in a very fine truncation beset with papillae.

Alimentary canal. The alimentary canal runs from the extreme anterior end to within a short distance of the posterior end. The mouth is situated in the centre of the blunt anterior end of the body. Six grooves radiate from the mouth outwards, one dorsally, one ventrally, and two on each side (Pl. III, fig. 4). The mouth leads into the pharynx, a narrow straight channel with thick walls which swell out and form the bulb, characteristic of nematodes; in the centre of this a slight dilatation of the lumen occurs, in which the chitin is thickened, which gives it a gizzard-like appearance; but more probably the organ

is suctorial in function. The anterior part of the pharynx contains the spear, a thick chitinous perforated needle which is bounded posteriorly by a distinct rim which is possibly trilobed. The spear is protrusible and can be used as a spine for piercing the plant tissues and as a tube through which the juices on which the animal feeds can be sucked (Pl. III, fig. 3). Posterior to the swelling the alimentary canal passes back almost straight to the anus. At first it is narrow, and may be termed an oesophagus, or it may be part of the chitinous pharynx. Anyhow, this soon widens out into the intestine (Pl. III, fig. 1). All the above parts appear to be more or less muscular, and are concerned with the inhibition of the plant's juices, and with the retraction of the spear The protraction muscles are a set running from the rimmed base of the spear to the thickened chitinous areas around the mouth (Pl. III, fig. 3). The walls of the intestinal part suggest no muscular layer, but thicken and present an irregular almost lobular condition outwardly, but a smooth and straighter margin internally bounding the gut cavity. Nuclei can be seen here and there, but are recognisable only with difficulty. They are small and spherical. The walls contain alternately clear spaces, and parts dense with granules which darken under the influence of the osmic acid and so probably are of a fatty nature. The terminal part —or rectum —is devoid of these granules, and the walls are thin. In the male the rectum opens by the anus into the anterior end of a shallow cloacal depression. In the female there is no distinct cloaca.

The intestinal part of the alimentary canal is composed of comparatively few cells, and probably not more than two or three would be seen in an ordinarily thin transverse section.

The body wall. The cuticle which covers the body wall has already been described. The cellular part of the body wall consists of a thin layer of protoplasm in which nuclei lie arranged fairly regularly. In stained specimens these nuclei are easily distinguishable from those of the alimentary canal by their much larger size. On treatment with Hermann's fluid the body wall nuclei are quite easily visible (Pl. III, fig. 6). The inner surface of the body wall bounding the body cavity is uneven and is especially thickened just posterior to the suctorial bulb where also it is composed of a protoplasm denser than elsewhere.

In the male a thickening of the body wall occurs upon the ventral surface of the tail, that is to say between the terminal point and the posterior edge of the cloaca (Pl. III, fig. 7). It is probably a special muscular development in connection with the wall of the cloaca to

which the spicules are attached, but the actual relations I have not been able to determine.

The body cavity, which in nematodes is probably not a true coelom, extends from end to end of the animal and can easily be made out in optical section. I have not been able to see any corpuscles or other bodies floating in it. There are no cilia. The alimentary canal and reproduction organs hang freely in the cavity unattached by any mesentery, and connected only near their openings and by a few muscular strands in the case of the former organ at the anterior end (Pl. III, figs. 5 and 6).

Reproduction organs. The sexes are separate.

The male organs consist of the reproduction gland, its duct and certain accessory cuticular organs already mentioned in the description of the whole animal. The reproduction gland is a simple elongated organ, the posterior end of which is tubular. This tube opens into the cloacal depression near its anterior margin. The anterior end of the organ which is solid and may be called a testis projects freely forward to a point about three-fifths the distance from the mouth to the anus. It lies usually upon the right side of the alimentary canal

At the extreme anterior end two kinds of cells can be distinguished, smaller laterally placed cells which are probably follicular (Pl. III, fig. 6) and larger more centrally placed cells which are spermatogonia. In tracing the reproduction gland backwards towards its tubular posterior continuation one can follow the development of the spermatogonia into the resting or growth stage of spermatocyte I. These are very large cells each of which occupies the full width of a follicle. Each contains one large nucleus in the resting phase. Then comes a series of smaller cells which are no doubt the products of the division of spermatocyte I and so may be called spermatocyte II. They no longer shew a resting nucleus but chromatin grains can be seen indistinctly. The difficulty of staining has prevented the determination of the details of the meiotic phase which no doubt occurs here. The subsequent division of spermatocytes II into spermatids is not distinguishable; but the lowest cells in the tube are almost certainly spermatids. If this is the correct interpretation, then the division of the chromosomes of spermatocyte II follows the meiotic stage immediately without any intervening resting stage, as no distinct nucleus can be seen after the growth period of spermatocyte I.

The final formation of the spermatozoon (which is non-flagellate) takes place at the posterior end, and the nearly mature sex cells are

seen in (Pl. III, fig. 1) as oblong masses of protoplasm arranged in a single row.

The thin walled tube—the vas deferens—which runs backwards as a wide thin walled duct from this point opens to the exterior just anterior and ventral to the rectum at the anterior end of the cloaca (Pl. III, fig. 1).

In connection with the male organs the two chitinous spicules mentioned above may be described here more carefully.

The spicules are curved chitinous organs, probably developed as local thickenings of the general chitinous covering of the body. They are placed in a special pocket of the cloaca (Pl. III, fig. 7), and are attached to the base of this pocket. The spicules are two in number placed right and left. When viewed in the ordinary way from the side only one of the pair can be seen. Each spicule, however, consists of two parts—an anterior smaller and a posterior much stouter part, joined by a very firm curved rod (Pl. III, fig. 8), so that when seen from the side the appearance is that of two, one in front of the other. The exact nature and position of these spicules is a matter of some importance in determining the genus and species and so it is advisable to give a detailed description of them. The stouter posterior limb is curved as in the figure and tapers towards its distal end. It is flattened from side to side. Its posterior end is truncated and oblique and from the posterior or upper margin the slender bar runs which joins it to the ventral or anterior limb. This is also curved, but is much smaller and much thinner. I think there is no connecting piece between the right and left spicules. In their natural position the proximal or basal ends are far apart, and probably are one on each side of the cloaca, but the distal or terminal ends of the stout limbs are in contact with one another. I think the anterior slender limbs are not in contact, but of this I am not sure.

In some other members of Anguillulidae, the spicules are described as having accessory pieces which lie *posterior* to the spicules themselves, to which the spicules may or may not be attached. In all cases the accessory (posterior) pieces are smaller than the spicules themselves.

In this species are we to take the stout limbs to be the spicules, and the other pieces modifications of them, or should we consider them as accessory pieces to which they have become fused? If so, then the accessory pieces are anterior instead of posterior. Or may we regard the stout limbs as being really the homologue of the accessory pieces,

but which have become enlarged so as to be more important than their principals?

The female organ which is rather more difficult to observe is also single. It lies more dorsal to the alimentary canal anteriorly, but its hinder part and the duct to the exterior lie to the right of the intestine. The actual opening of the oviduct is, I think, median upon the ventral surface. Its edge is uneven, almost serrated (Pl. III, fig. 5). It differs from the male organ in two well-marked respects.

(*a*) Its opening to the exterior is some way anterior to the opening of the alimentary canal, although still in the hinder part of the animal.

(*b*) A blind pocket projects backwards behind the opening of the duct which may be called a receptaculum ovarum as it seems to contain ripe ova. Probably fertilisation takes place here and possibly some part of development may be undergone here, in which case it would be termed more correctly a uterus, but I have not so far seen any advanced embryos or even segmental ova in it.

The extreme anterior end of the organ is very like the corresponding part of the male gland. It contains follicle cells and oogonia. As in the case of the male one can see as one looks along the gland that in the lower parts the oogonia pass into large oocytes (Pl. III, fig. 2). There is then a region in which all trace of a large nucleus is absent and the cells are much crowded. This is no doubt the region in which the meiotic phase is taking place, and is followed by a string of rounded cells which again shew a nucleus. This I take to be the oocyte II stage. Close to the lower end of the oviduct, just before the opening, a second region in which the nuclei are absent probably represents the last maturation stage or heterotype division, beyond which a few "ova" may be seen. These seem to pass over the opening of the oviduct into the uterus.

There is another possible interpretation. Are the cells in this so-called receptaculum ovarum or uterus really spermatozoa? In this case perhaps the ova do not pass into this pocket at all, and the pocket is therefore a receptaculum seminis.

A further question then arises—is this pocket really a diverticulum of the oviduct or is it a depression in a surface (as is often the case in receptacula seminii, *e.g.*, oligochaeta)? If so, is it homologous with the depression in which the spicules of the male are situated? .

Nervous system. Very little has been made out about this. I can find in some cases a band of tissue running across the oesophagus which may very likely be a nerve collar.

Excretory system. I have not been able to see anything of this.

To what species or genus are we to ascribe this black currant nematode?

Some years ago correspondence was undertaken with Professor J. Ritzema Bos and Dr G. de Man to both of whom, as specialists in this group of nematodes, specimens of the species described above were sent. They kindly examined the specimens but neither was able to say definitely whether the species was a form undescribed hitherto or not, but both agreed that it should be placed in the genus Aphelenchus. The chief difficulty which presented itself to Dr G. de Man was the insufficient description of the species already described. Although much remains still to be determined, I have been able to clear up a good many points in my description given above which I hope may be useful to other workers in this field.

The species with which Dr G. de Man was inclined to identify this black currant nematode is *A. fragariae*; but quite apart from what seem to be anatomical differences the facts given on p. 264, with regard to the life history and habits of the two forms, make the identity extremely improbable.

I would suggest that the species does not belong to the genus Aphelenchus at all.

Professor Bos says of the reproduction organ of the female *A. fragariae* "that the ovary is double, one lies in front of the genital opening the other behind." It is possible that the condition is really as described above for the black currant species, and that the hindwardly directed tube is not an ovary, but a receptaculum seminis

Ritzema Bos also describes *A. ormerodis*, a smaller species than *A fragariae*, also found on the strawberry plant and causing disease. In one particular this species may be more like the black currant species. This is the spicule. It is drawn as a slightly curved spicule with a much smaller accessory piece quite unattached to it in front. "The spicules are rather large, more developed than in *A. fragariae*: also I found here an accessory piece." It is possible that this description is not accurate, but it is something like the idea which one receives from a cursory glance at the spicules of the black currant species. If it is accurate, then clearly the two species differ considerably. Here again experiment failed to establish the black currant species upon the strawberry plant.

To sum up:

The reasons against the identity of this species with *A. fragariae* are

(a) the failure to get it to produce the characteristic symptoms of attack on the strawberry plant;

(b) the totally different character of the spicules;

(c) the absence of sudden reduction in diameter behind the cloaca;

(d) large size of the spear;

(e) the restricted extension forwards of the testis—which in *A. fragariae* reaches to the anterior third of the length of the body and in the black currant species does not extend beyond the middle;

(f) the swollen lips of the truncated end.

The reasons against identification with *A. ormerodis* are

(a) the failure to infect the strawberry plant with this species;

(b) the larger size of the black currant species—·92 mm. as against ·65 mm. for large specimens in each case;

(c) the difference in the character of the spicules, though this may be more apparent than real (v. above);

(d) the presence of the narrow pharyngeal region (oesophagus) between the bulb and true intestine;

(e) the restricted extension forwards of the testis;

(f) the swollen "lips" of the truncated anterior end.

Although the black currant worm may be said to possess characters which belong to both the genus Aphelenchus and Tylenchus, yet it has more in common with the latter group than the former. Especially is this the case if the thin ridges alongside the cloaca can be interpreted as a bursa.

It is therefore considered advisable, until further work has been done on these groups, to include the worm in question in the genus Tylenchus with the specific name of ribes.

DESCRIPTION OF PLATE III.

Fig. 1. Male. Fig. 2. Female.
„ 3. Mouth parts enlarged. „ 4. Anterior opening.
„ 5. Portion of reproductive organs of ♀.
6. Portion of reproductive organs of ♂.
7. Spicule of ♂ from side.
8. Spicule of ♂ (higher magnification shewing complete spicule).
9. Cloaca of ♂ (spicule omitted).
10. Terminal papillae of ♂.

Al.c. alimentary canal; *c.al.c.* cavity of alimentary canal; *b.c.* body cavity; *b.w.* body wall; *cl.* cloaca; *f.c.* follicle cell; *f.w.* follicle wall; *n.* nucleus; *n.b.w.* nucleus of body wall; *ooc.* oocyte; *oog.* oogonia; *ov.* ovary; *ovd.* oviduct, *o.ovd.* opening of oviduct; *ph.* muscular pharynx; *pp.* papillae; *p.spg.* primary spermatogonia; *r.s.* receptaculum seminis, *sp.* spine; *spc.* spermatocyte; *spg.* spermatogonium; *spt.* spermatid; *spl.* spicule, *sp.m.* special muscle; *spz.* spermatozoa; *v.d.* vas deferens, *w.ph.* wall of pharynx.
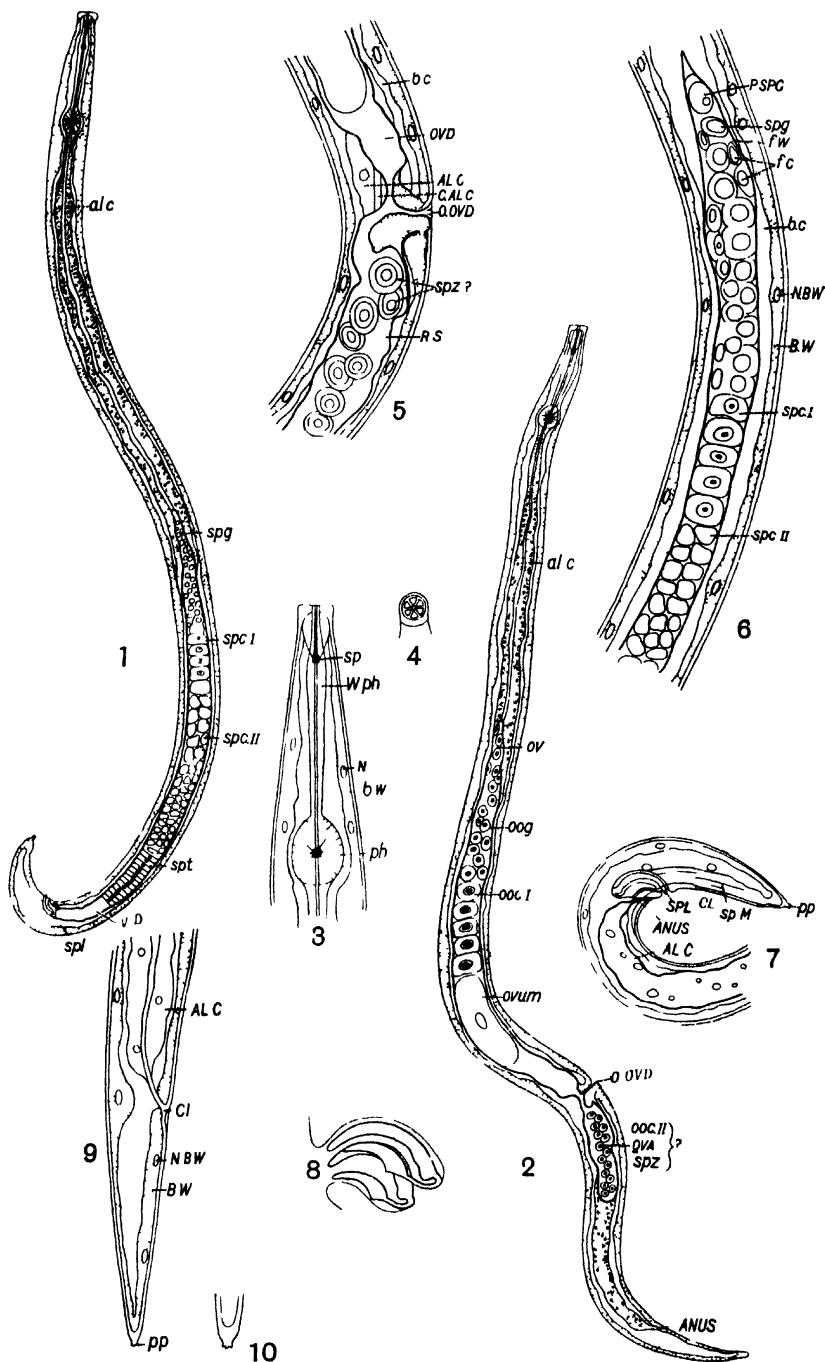
THE SOLUBILITY OF CALCIUM PHOSPHATES IN CITRIC ACID.

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MODERN writers on theoretical chemistry and particularly those who have written on Agricultural Chemistry, have either stated directly or have in other cases implied, that, excluding basic slag, calcium and phosphoric acid exist in three states of combination, namely mono-, di- and tricalcic phosphate, and that the former is soluble in water, the second is insoluble in water but soluble in ammonium citrate (or citric acid), while the third form is insoluble both in water and in ammonium citrate (or citric acid). That this is so is further confirmed by the general acceptance of the use of ammonium citrate solution, to differentiate between "reverted phosphate" (dicalcic phosphate) and tricalcic phosphate. On this matter Aikman[1] writes: "value of reverted phosphate....At first it was thought that it was impossible to estimate its quantity by chemical means. This difficulty has been overcome and it is generally admitted that the ammonium citrate process furnishes an accurate means of determining its amount." The above statement implies that the ammonium citrate dissolves the dicalcic phosphate and that the tricalcic phosphate is insoluble in ammonium citrate. In Thorpe's *Dictionary of Applied Chemistry*[2] the following statement occurs: "Phosphates that are soluble in ammonium citrate may be safely regarded as assimilable by plants; in America they are regarded as of almost equal value with water soluble phosphate; that they are so always is certainly open to doubt. Phosphates that are insoluble in ammonium citrate are often effective as manure. Ammonium citrate thus gives no safe distinction between assimilable and non-assimilable phosphates, though it affords a useful approximate means of determining

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[1] Aikman, *Manures and Manuring*, p. 391.

[2] Thorpe, *Dictionary of Applied Chemistry* (latest edition), Vol. II, p. 549.

'reverted' phosphate in superphosphate. As a measure of ready availability in basic slag a 2 % solution of citric acid has now superseded ammonium citrate."

To test some of these statements, and to add to our knowledge on the subject, it was decided to purchase or to prepare Tricalcium phosphate, and then to ascertain its behaviour towards the prescribed citric acid solution. Citric acid was chosen, as in modern practice this solvent has taken the place of ammonium citrate, and the results are similar so far as my experiments have gone.

In the analytical work, connected with the experiments about to be described, lime had been determined by three methods:

(*a*) Adding sulphuric acid and evaporating till a considerable crop of crystals of gypsum has formed. Cool, add alcohol, filter, wash, ignite, and weigh as $CaSO_4$[1].

(*b*) Dissolve in hydrochloric acid and add ammonia till precipitate just begins to form. Redissolve this in a drop of hydrochloric acid, add ammonium oxalate in excess and then acetate of soda. Allow to settle, filter, wash, dry, ignite, and weigh as CaO[2]. I find that when this method is followed, the lime precipitate always contains minute quantities of phosphoric acid, which is greater the larger the amount of lime used and precipitated. For accurate determinations this must be determined and deducted. It has been found preferable to precipitate the lime with excess of ammonium oxalate in a solution to which acetic acid has been added. When this is done mere traces of phosphoric acid occur in the lime precipitate.

(*c*) Girard's method or Reynoso's modification of this, of removing the phosphoric acid in nitric acid solution by means of tin-foil, filtering and precipitating the lime by ammonium oxalate[3]. It was found that the filtrate from the stannic acid and phosphoric acid compound contains small amounts of tin which must be removed by sulphuretted hydrogen before precipitating the lime. Phosphoric acid has been determined by precipitating with molybdate reagent. Dissolving the precipitate in ammonia and precipitating with magnesia mixture, following the method adopted in The Fertilisers and Feeding-stuffs (Methods of Analysis) Regulations 1908[4].

[1] Wiley, *Principles and practice of Agricultural Analysis*, Vol. II, p. 21.

[2] Fresenius, *Quantitative Chemical Analysis*, Vol. I, p. 188.

[3] Crookes, *Select methods of Chemical Analysis*, p. 499.

[4] *Fertilisers and Feeding Stuffs Regulations*, 1908. Board of Agriculture. Fisheries Leaflet No. 18, p. 17.

The calculations involved are based upon the following considerations: dicalcic phosphate contains 1 part CaO ·1607 parts water of crystallisation, 1·26786 parts P_2O_5; tricalcic phosphate contains 1 part CaO with ·84524 parts P_2O_5; calcium hydrate contains 1 part CaO with ·3214 parts water.

In mixtures containing di- and tricalcic phosphates, when the total CaO and total P_2O_5 are determined the amounts of di- and triphosphate is calculated as follows:

Let $x = $ CaO existing as dicalcic phosphate,

and $y = $ CaO existing as tricalcic phosphate;

then $x \times 1·26786 = P_2O_5$ existing as dicalcic phosphate,

and $y \times ·84524 = P_2O_5$ existing as tricalcic phosphate.

$$1·26786x + ·84524y = \text{total phosphoric acid found} \quad \dots\dots\dots\dots(1),$$

$$x + y = \text{total lime found} \quad \dots\dots\dots\dots\dots\dots(2).$$

Solving these equations the values of x and y are obtained.

In mixtures containing dicalcic phosphate ($CaHPO_4 2H_2O$), tricalcic phosphate ($Ca_3P_2O_8$) and calcium hydrate ($CaOH_2O$), having determined the total CaO, total P_2O_5 and water, the following is the method adopted:

Let $x = $ CaO as dicalcic phosphate, $y = $ CaO as tricalcic phosphate,

$z = $ CaO as calcium hydrate.

$$·1607x + ·6428y + ·3214z = \text{total water determined} \quad \dots\dots\dots\dots(1),$$

$$x + y + z = \text{total lime determined} \quad \dots\dots\dots\dots(2),$$

$$1·26786x + ·84524y = \text{total } P_2O_5 \text{ determined} \quad \dots\dots\dots\dots(3).$$

From (1) obtain value of z in terms of x and substitute in (2), equate this with (3) and obtain value of x. y and z are then found.

The following three samples, Nos. 1, 2, and 3, of phosphate of lime were obtained by purchase.

No. 1. Phosphate of lime in a 1 lb. bottle and of English manufacture.

This sample was found by analysis to contain:

Lime (CaO) ...	...	...	...	...	48·26
Phosphoric acid (P_2O_5)	...	...	...	...	43·26
Water ...	...	...	...	...	8·48
					$\overline{100·00}$

	Per cent.
Loss in weight on drying in desiccator over sulphuric acid in 16 hours ...	1·036
Loss in weight on drying in desiccator over sulphuric acid in 112 hours ...	1·620
Loss on drying at 100° C. in 2 hours, 2·37 % in 4 hours	2·37
Loss on ignition	8·47

By calculation the lime and phosphoric acid exist as:

	CaO	H_2O	P_2O_5	Water	
Dicalcic phosphate $(CaO)_2H_2OP_2O_5$	5·842	0·939	7·407	—	= 14·188
Tricalcic phosphate $(CaO)_3P_2O_5$ ...	42·418	—	35·853	—	= 78·271
Water of crystallisation	—	—	—	7·541	= 7·541
	48·260	0·939	43·260	7·541	100·000

If the water of crystallisation present be contained in the dicalcic phosphate only and none with the tricalcic phosphate we would have

	CaO	H_2O	P_2O_5	Water (theory require 7·512)	
Dicalcic phosphate $(CaO)_2H_2OP_2O_5 8H_2O$	5·842	0·939	7·407	7·541	= 21·729
$CaHPO_4 . 4H_2O$					
Tricalcic phosphate $Ca_3P_2O_8$ ...	42·418	—	35·853	—	78·271
	48·260	0·939	43·260	7·541	100·000

But as the tricalcic phosphate might also contain some water of crystallisation, the first statement is probably more correct.

The above sample therefore appears to be a mixture of 14·188 % di- and 78·271 % tricalcic phosphate with water of crystallisation.

No. 2.   Calcii Phosphas B.P., purchased locally.
Upon analysis the following figures were obtained:

Lime (CaO)	40·15
Phosphoric acid (P_2O_5)	47·52
Water	12·33
	100·00

Per cent

Loss in weight on drying in desiccator over sulphuric acid for 16 hours	1·184
Loss in weight on drying in desiccator over sulphuric acid for 112 hours	1·620
Loss on drying at 100° C.: 2 hours 3·46 %, 6 hours 3·51 %. 9 hours ...	3·60
Loss on ignition	12·35

By calculation the lime and phosphoric acid are found to exist in the following combination:

	CaO	H_2O	P_2O_5	Water	
Dicalcic phosphate $(CaO)_2H_2OP_2O_5$	32·142	5·166	40·751	—	= 78·059
Tricalcic phosphate $(CaO)_3P_2O_5$ ...	8·008	—	6·767	—	= 14·777
Water of crystallisation	—	—	—	7·164	= 7·164
	40·150	5·166	47·518	7·164	= 100·000

If all the water of crystallisation be contained in the dicalcic phosphate only, the dicalcic phosphate present would conform to the formula

$(CaO)_2H_2OP_2O_5$ 1·4 H_2O or $CaHPO_4$ 0·7 H_2O. This preparation therefore appears to be a mixture of 78·059 % dicalcic phosphate and 14·777 % tricalcic phosphate with water of crystallisation.

No. 3. Calcii Phosphas B.P., also purchased locally.
Upon analysis the following figures were obtained:

Lime (CaO) ...	...	...	...	41·15
Phosphoric acid (P_2O_5)	...	...	42·20
Water	...	...	...	16·65
				100·00

	Per cent.
Loss in weight on drying in desiccator over sulphuric acid 16 hours	1·920
Loss in weight on drying in desiccator over sulphuric acid 112 hours	2·760
Loss on drying at 100° C.: 2 hours 5·38 %, 4 hours 5·38 %, 6 hours 5·45 %, 11 hours	5·52
Loss on ignition	16·66

By calculation we find the lime and phosphoric acid exist in the following combination:

	CaO	H_2O	P_2O_5	Water	
Dicalcic phosphate ($CaO)_2H_2OP_2O_5$	17·554	2·821	22·256	—	= 42·631
Tricalcic phosphate ($CaO)_3P_2O_5$ ...	23·596	—	19·944	—	= 43·540
Water of crystallisation	—	—	—	13·829	= 13·829
	41·150	2·821	42·200	13·829	100·000

Assuming that all the water of crystallisation is associated with the dicalcic phosphate only, this would conform to the formula

$$(CaO)_2H_2OP_2O_54·9H_2O \quad \text{or} \quad CaHPO_42·45H_2O.$$
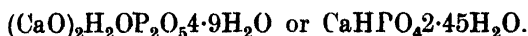
The above sample therefore appears to be a mixture of 42·631 % dicalcic phosphate with 43·540 % tricalcic phosphate with water of crystallisation.

None of these three compounds are what they purport to be, but are mixtures of varying amounts of di- and tricalcic phosphates.

Having been unsuccessful in procuring pure tricalcium phosphate by purchase, the preparation of this substance was undertaken, following recognised authorities.

Roscoe and Schorlemmer[1] state: "pure calcium phosphate $Ca_3P_2O_8$ is obtained as a white precipitate by adding an excess of common sodium phosphate to an ammoniacal solution of chloride of calcium." This method was adopted by Warington[2] in preparing pure tricalcic phosphate for his investigations on the action of water on this substance.

[1] *Treatise on Chemistry*, Vol. II, Part I, p. 205.
[2] "On the decomposition of tricalcium phosphate by water," *Journal Chemical Society*, Vol. XXVI (entire series), p. 983.

The method was followed and the product obtained was marked as phosphate No. 4.

On submitting this product (No. 4) to chemical analysis the following data was obtained:

Lime (CaO)	52·93
Phosphoric Acid (P_2O_5)	41·44
Water (combination and constitution) ...	5·68
	100·05

	Per cent.
Loss on drying at 100° C. in 2 hours	1·91
Loss on drying at 100° C. in 6 hours	1·95
Loss on ignition	5·68

By calculation the lime and phosphoric acid probably exist as:

		CaO	P_2O_5
Dicalcic phosphate	... ...	4·590	5·819
Tricalcic phosphate	... ...	42·142	35·621
Lime (free)	... ...	6·198	—
		52·930	41·440

Assuming that the water present is associated only with the dicalcic phosphate and with the lime as hydrate, the formula of the dicalcic phosphate present is $(CaO)_2H_2OP_2O_54H_2O$ or $CaHPO_42H_2O$. Thus:

	CaO	Water of constitution	P_2O_5	Water of crystallisation	
Dicalcic phosphate $(CaO)_2H_2OP_2O_54H_2O$	4·590	0·738	5·819	2·950	= 14·097
Tricalcic phosphate $(CaO)_3P_2O_5$ ...	42·142	—	35·621	—	77·763
Calcium hydrate $CaOH_2O$	6·198	1·992	—	—	8·190
	52·930	2·730	41·440	2·950	=100·050

This preparation therefore is not pure tricalcic phosphate, but is a mixture of 77·76 % tricalcic phosphate, 14·10 % dicalcic phosphate and 8·19 % calcium hydrate.

The method given in the *British Pharmacopœia*[1] for the preparation of tricalcium phosphate, by dissolving bone ash in hydrochloric acid and precipitating with ammonium hydrate was next tried.

The preparation thus obtained was marked No. 5, and upon analysis gave the following data:

Lime (CaO)	53·05
Phosphoric acid (P_2O_5)	41·17
Water	5·78
	100·00

[1] *The British Pharmacopœia* (Spottiswoode and Co., London), 1891, p. 87.

By calculation the lime and phosphoric acid would probably exist as:

	CaO	P_2O_5
Dicalcic phosphate ..	4·547	5·765
Tricalcic phosphate ...	41·887	35·405
Lime (free)	6·616	—
	53·050	41·170

Assuming that the water present is associated only with the dicalcic phosphate, and with the lime as hydrate, the formula of the dicalcic phosphate present would be $(CaO)_2H_2OP_2O_54H_2O$ or $CaHPO_42H_2O$.

Thus:

	CaO	Water of constitution	P_2O_5	Water of crystallisation	
Dicalcic phosphate $(CaO)_2H_2OP_2O_54H_2O$ ...	4·547	0·731	5·765	2·923	= 13·966
Tricalcic phosphate $(CaO)_3P_2O_5$	41·887	—	35·405	—	= 77·292
Calcium hydrate $CaOH_2O$ ...	6·616	2·126	—	—	= 8·742
	53·050	2·857	41·170	2·923	= 100·000

This preparation is not pure tricalcic phosphate, but is a mixture of 77·29 % tricalcium phosphate, 13·97 % bicalcium phosphate and 8·74 % calcium hydrate.

A second attempt was made to prepare pure tricalcium phosphate by dissolving bone ash in hydrochloric acid and precipitating with ammonium hydrate, following the method of the *British Pharmacopœia*, but washing the precipitate till free from chlorides with very dilute ammonium hydrate as was done in the old analytical method of determining phosphoric acid as tricalcic phosphate.

The product obtained after drying and igniting was marked No. 6. Upon analysis the product was found to consist of:

Lime (CaO)	56·15
Phosphoric acid (P_2O_5)	43·85
	100·00

Since the above is most probably a mixture of di- and tricalcic phosphate with free lime, it is impossible from the data given to calculate the percentage of each, since several such mixtures could be calculated having 56·15 % CaO and 43·85 % P_2O_5.

Since 43·85 P_2O_5 requires 51·997 CaO to form tricalcic phosphate, and 34·664 CaO to form dicalcic phosphate, the free lime may be anything between 56·15 minus 51·997 = 4·153 and 56·15 minus 34·664 = 21·486. If however the free lime be 7·00 % (which is about that

present in the hydrated product previously discussed (No. 5) we would have:

	CaO	P₂O₅	
Dicalcic phosphate ...	5·46	6·92	= 12·38
Tricalcic phosphate...	43·69	36·93	= 80·62
Lime (free)	7·00	—	= 7·00
	56·15	43·85	=100·00

When time permits I hope to investigate the question of the estimation of the free lime actually present in such a mixture as the above, but it is evident that the product obtained by this method is not tricalcic phosphate as is usually stated and as it is assumed to be.

Experiments were next made to prepare tri- and dicalcic phosphates from pure phosphoric acid (H_3PO_4) by the addition of three equivalents and of two equivalents respectively of lime (CaO). The solution of phosphoric acid used was prepared by taking about 32 grams pure phosphorus pentoxide, dissolving in water and making up to 500 c.c. Gravimetric analysis showed that the 500 c.c. solution contained 31·66 grams phosphoric acid (P_2O_5).

Tricalcium phosphate preparation No. 7.

For the preparation of this 243 c.c. of the above mentioned solution of phosphoric acid containing 15·386 grams P_2O_5 were taken and to this was added 18·203 grams pure freshly ignited lime (CaO). A further small quantity of lime was added in the form of lime-water, the idea being to have a faint excess of lime— which excess could be easily washed out by water. Owing to an absence from Sydney, the precipitate formed was not separated from the liquid portion for about a fortnight after the addition of the lime to the phosphoric acid.

This precipitate when dried weighed 36·22 grams, and was marked preparation No. 7. The filtrate and washings contained lime but no phosphoric acid and measured 1000 c.c. On evaporation, the filtrate and washings yielded 0·53 gram lime (CaO).

Dicalcium phosphate preparation No. 8.

To 243 c.c. of the phosphoric acid solution mentioned above containing 15·386 grams P_2O_5, 12·135 grams pure lime (CaO) (equivalent to 2 molecules of lime) were added.

This mixture also stood for a fortnight before filtering. 19·63 grams dried preparation (No. 8) were obtained.

The filtrate (without the washings) which measured about 500 c.c. was evaporated. When boiling commenced, the clear fluid became milky, and a precipitate separated out. Evaporation was continued

till bumping was severe. The precipitate was filtered off, washed well with water, and on drying weighed 2·210 grams. This was marked preparation 8A. The filtrate from which the last mentioned preparation (8A) was obtained, was further evaporated on the water bath to small bulk but not to dryness. On standing for two days the mass crystallised. This mass was placed on a filter paper and washed with water. The washings contained much lime and phosphoric acid. The filter paper and contents were placed in a water oven to dry but the paper blackened and then charred. This last product therefore appears to be an acid calcium phosphate; the weight would be about 1·1 gram.

The result of the chemical analyses of these various preparations will now be given.

Preparation No. 7 (3 equivalents CaO: 1 equivalent P_2O_5) was found to consist of:

Lime (CaO)	48·61
Phosphoric acid (P_2O_5)	42·48
Water	8·91
	100·00

By calculation the above numbers correspond to a mixture of:

	CaO	H_2O	P_2O_5	Water	
Dicalcic phosphate $(CaO)_2H_2OP_2O_5$...	3·297	0·530	4·180	—	= 8·007
Tricalcic phosphate $(CaO)_3P_2O_5$ ...	45·313	—	38·300	—	= 83·613
Water of crystallisation (H_2O) ...	—	—	—	8·380	= 8·380
	48·610	0·530	42 480	8·380	=100·000

If all the water of crystallisation be associated with the dicalcic phosphate, the composition of this would correspond to the formula $(CaO)_2 . H_2O . P_2O_5 . 15·8H_2O$ or $CaHPO_4 7·9H_2O$.

If as is more probable, the water of crystallisation is shared by both di- and tricalcic phosphates, and assuming that $CaHPO_4 2H_2O$ is the particular form of dicalcic salt present, then the formula of the tricalcic phosphate present is $(CaO)_3P_2O_5 1·29H_2O$. . Thus:

Dicalcic phosphate $(CaO)_2H_2OP_2O_54H_2O$ ...	10·127
Tricalcic phosphate $(CaO)_3P_2O_51·29H_2O$ ...	89·873
	100·000

Preparation No. 8 (2 equivalents of CaO : 1 equivalent P_2O_5) was found to consist of:

Lime (CaO)	46·36
Phosphoric acid (P_2O_5)	41·77
Water	11·87
	100·00

By calculation the above appears to consist of:

	CaO	H_2O	P_2O_5	Water	
Dicalcic phosphate $(CaO)_2H_2OP_2O_5$	6·116	0·983	7·754	—	= 14·853
Tricalcic phosphate $(CaO)_3P_2O_5$ ...	40·244	—	34·016	—	= 74·260
Water of crystallisation	—	—	—	10·887	= 10·887
	46·360	0·983	41·770	10·887	= 100·000

If the whole of the water of crystallisation be contained in the dicalcic phosphate only, then the formula of this would correspond to $(CaO)_2H_2OP_2O_5 11H_2O$, but if, as is more probable both the di- and tri-salts contain water of crystallisation, and if the di-phosphate be $CaHPO_4 2H_2O$ the tricalcic phosphate would correspond to the formula $Ca_3P_2O_8 1·61H_2O$. Thus:

$$(CaO)_2H_2OP_2O_5 4H_2O \quad ... \quad ... \quad 18·785$$
$$(CaO)_3P_2O_5 1·61H_2O \quad ... \quad ... \quad 81·215$$
$$\overline{100·000}$$

Preparation No. 8A was found upon analysis to consist of:

$$Lime (CaO) ... \quad ... \quad ... \quad ... \quad 41·386$$
$$Phosphoric\ acid\ (P_2O_5) \quad ... \quad ... \quad 50·596$$
$$Water \quad ... \quad ... \quad ... \quad ... \quad 8·018$$
$$\overline{100·000}$$

By calculation the above appears to be a mixture of:

	CaO	H_2O	P_2O_5	Water	
Dicalcic phosphate ...	36·949	5·938	46·846	—	= 89·733
Tricalcic phosphate ...	4·437	—	3·750	—	= 8·187
Water of crystallisation ...	—	—	—	2·080	= 2·080
	41·386	5·938	50·596	2·080	= 100·000

If all the water of crystallisation be associated with the dicalcic phosphate only, its formula would correspond to $(CaO)_2H_2OP_2O_5 0·35H_2O$. The tricalcic phosphate would be $Ca_3P_2O_8$.

It was considered probable that in the last experiments (preparations No. 7 and No. 8) hydrolysis of the salts originally produced might easily have occurred, owing to the lapse of time between the mixing of the lime and phosphoric acid, and the separation of the precipitates formed, from the fluid portion. It was therefore decided to repeat this portion of the work, and to remove the precipitate formed after the lapse of an hour. Preparation of the solution of phosphoric acid (H_3PO_4):

About 43 grams pure phosphorus pentoxide was dissolved in water and made up to 500 c.c.

A titration of this to methyl-orange, using caustic soda, gave

41·54 grams P_2O_5. Quadruplicate gravimetric determinations gave 41·664 grams P_2O_5 present in the 500 c.c. solution.

Preparation of tricalcic phosphate from 3 equivalents CaO to 1 equivalent P_2O_5 preparation No. 9.

225 c.c. of the above phosphoric acid solution containing 18·745 grams P_2O_5 were taken. 22·1796 grams pure freshly ignited lime prepared by igniting pure calcium carbonate was weighed out. About 100 c.c. of water was added to the lime, then the phosphoric acid added, stirred well, and allowed to stand with frequent stirring for one hour.

The precipitate was filtered off and dried at 100° C.

Product obtained by mixing 2 equivalents of CaO with 1 equivalent P_2O_5 preparation No. 10.

11·2644 grams pure lime prepared as above described was weighed out. To this was added about 100 c.c. water, and 171·5 c.c. phosphoric acid solution containing 14·2816 grams P_2O_5. The mixture was well stirred at frequent intervals during an hour. At the end of that time the precipitate was filtered off, washed well with water, and dried at 100° C. The weight of the product obtained was 20·74 grams.

Upon analysis preparation No. 9 was found to contain:

Lime (CaO)	50·12
Phosphoric acid (P_2O_5)	42·36
Water	7·52
	100·00

By calculation the above lime and phosphoric acid corresponds to $3CaO : P_2O_5$ or pure tricalcium phosphate. The water of crystallisation present indicates that the formula of the tricalcic phosphate is $Ca_3P_2O_8 1·384H_2O$ or $8Ca_3P_2O_8 11H_2O$.

This preparation when dried at 125° C. has apparently the composition of $Ca_3P_2O_8 1·3H_2O$ or $10Ca_3P_2O_8 13H_2O$.

The analysis of preparation No. 10 (from 2 equivalents CaO to 1 equivalent P_2O_5) showed this product to consist of:

Lime (CaO)	44·58
Phosphoric acid (P_2O_5)	44·30
Water	11·12
	100·00

By calculation the above numbers are found to indicate a mixture of di- and tricalcic phosphate. Thus:

		CaO	H₂O	P₂O₅	Water	
Dicalcic phosphate	...	15·663	2·517	19·858	—	= 38·038
Tricalcic phosphate	...	28·917	—	24·442	—	= 53·359
Water of crystallisation	...	—	—	—	8·603	= 8·603
		44·580	2·517	44·300	8·603	=100·000

The water of crystallisation present appears to indicate that the dicalcic phosphate present corresponds to $(CaO)_2H_2OP_2O_52H_2O$ or $CaHPO_4H_2O$, and of the tricalcic phosphate $Ca_3P_2O_8 . H_2O$. Thus:

	CaO	Water of constitution	P_2O_5	Water of crystallisation	
Dicalcic phosphate					
$(CaO)_2H_2OP_2O_5 2H_2O$ ...	15·663	2·517	19·858	5·034	= 43·072
Tricalcic phosphate $(CaO)_3P_2O_5H_2O$	28·097	—	24·442	3·097	= 56·456
	43·760	2·517	44·300	8·131	= 99·528
	Water of crystallisation not allotted			...	0·472
					100·000

It appears, therefore, that the product formed by the interaction of 3 equivalents of lime (CaO) on 1 equivalent of phosphoric acid (P_2O_5) yields pure tricalcic phosphate when the precipitate formed is removed from the fluid portion within an hour of the commencement of the reaction. The tricalcic phosphate formed contains water of crystallisation. The amount of this is intermediate between that occurring in the mineral ornithite $Ca_3P_2O_82H_2O$, and collophanite $Ca_3P_2O_8H_2O$. When however 2 equivalents of lime (CaO) are added to 1 equivalent of phosphoric acid (P_2O_5) the resultant product is not dicalcic phosphate, but is a mixture of di- and tricalcic phosphates.

Having now obtained a pure tricalcium phosphate (preparation No. 9), experiments were carried out to ascertain what would be the result of treating (a) pure tricalcic phosphate, (b) tricalcic phosphate plus 25 % pure calcium carbonate, and (c) tricalcic phosphate plus 50 % pure calcium carbonate, with a 2 % citric acid solution in the manner prescribed for determining citrate soluble phosphoric acid[1]. Briefly stated, the method is to shake for 30 minutes in a rotary shaker making 30 to 40 revolutions per minute 5 grams of the substance and 500 c.c. of a 2 % citric acid solution.

In these experiments about to be described, the first extraction was made in the prescribed manner. The unexhausted phosphate remaining after the first extraction, was extracted a second time with a further quantity of 500 c.c. of a 2 % citric acid solution for a further period of 30 minutes. This formed the "second" extraction. A third and a fourth extraction was similarly made. At this stage the whole of the lime and the whole of the phosphoric acid had gone into solution.

Experiment (a). Extraction of 5 grams pure tricalcic phosphate containing 50·12 % CaO, 42·36 % P_2O_5, 7·52 % H_2O with 500 c.c. of

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[1] *Fertilisers and Feeding Stuffs Regulations*, 1908. Board of Agriculture. Fisheries Leaflet 18, p. 17.

a 2 % citric acid solution. The results are given in tabular form and are practically self-explanatory.

	Lime		Phosphoric acid		Lime calculated from P_2O_5 found to form with it $Ca_3P_2O_8$	Difference between calculated lime and that actually found
	Grams found calculated to 100 grams powder	Expressed in % of total lime present	Grams found calculated to 100 grams powder	Expressed in % of total P_2O_5 present		
1st extn, 30 min.	45·45	90·48	38·34	90·96	45·36	+0·09
2nd ,, 30 ,,	3·56	7·09	3·31	7·85	3·92	−0·36
3rd ,, 30 ,,	0·71	1·41	0·34	0·81	0·40	+0·31
4th ,, 30 ,,	0·51	1·02	0·16	0·38	0·19	+0·32
Total ...	50·23	100·00	42·15	100·00	49·87	+0·36
					50·23	
Actually present	50·12	—	42·36	—	—	—

It will be noted that 90·5 % of the lime and 91 % of the phosphoric acid have gone into solution in the first extraction of 30 minutes, and that the lime and phosphoric acid extracted are very nearly in the ratio of $3CaO : P_2O_5$.

In the second extraction it will be seen that there is rather less lime found than would be necessary to produce $Ca_3P_2O_8$ with the phosphoric acid found. This would appear to indicate that the lime and phosphoric acid had gone into solution as a mixture of di- and tricalcium phosphate.

In the third extraction, the lime found is greater than that which would be necessary to form $Ca_3P_2O_8$ with the P_2O_5 found, so that part of the lime found must have gone into solution as calcium citrate. This also appears to have taken place in the fourth extraction.

After four extractions the whole of the lime and the whole of the phosphoric acid have gone into solution.

It should also be noted that under existing methods of analysis this compound (tricalcic phosphate) would be stated to contain 38·34 % "citrate soluble" phosphoric acid out of a total of 42·36 %.

Experiment (b). Extraction of 5 grams of an intimate mixture consisting of 75 % pure tricalcic phosphate and 25 % pure calcium carbonate with 500 c.c. of 2 % citric acid solution.

	gram CaO	gram P_2O_5
3·75 grams tricalcic phosphate containing 50·12 % CaO, 42·36 % P_2O_5 will contain	1·8795	1·5885
1·25 grams calcium carbonate containing 56·00 % CaO, will contain	0·7000	—
	2·5795	1·5885

100 grams mixture ∴ contains $\begin{cases} 37\cdot59 \text{ grams CaO, } 31\cdot77 \text{ grams P}_2\text{O}_5 \text{ from the phosphate} \\ 14\cdot00 \text{ grams CaO} \end{cases}$ from the carbonate

$\overline{51\cdot59 \text{ grams CaO, } 31\cdot77 \text{ grams P}_2\text{O}_5}$

Note that in this mixture there is an excess of 14 % of lime.

	Lime		Phosphoric acid		Lime calculated from P$_2$O$_5$ found to form with it Ca$_3$P$_2$O$_8$	Difference between calculated lime and that actually found
	Grams found calculated to 100 grams mixture	Expressed in % of total lime present	Grams found calculated to 100 grams mixture	Expressed in % of total P$_2$O$_5$ present		
1st extn, 30 min.	44·80	86·82	26·85	84·44	31·77	+13·03
2nd „ 30 „	5·31	10·29	4·52	14·21	5·35	− 0·04
3rd „ 30 „	0·82	1·59	0·34	1·07	0·40	+ 0·42
4th „ 30 „	0·67	1·30	0·09	0·28	0·10	+ 0·57
Total ...	51·60	100·00	31·80	100·00	37·62	+13·98
					51·60	
Actually present	51·59	—	31·77	—	—	—

It will be noted that 86·8 % of the total lime and 84·4 % of the total phosphoric acid have gone into solution in the first extraction of 30 minutes, and that the lime and phosphoric acid extracted are not in the ratio of 3CaO : P$_2$O$_5$. The lime found is 13 grams more than that necessary to form Ca$_3$P$_2$O$_8$ with the P$_2$O$_5$ found, and this amount is very nearly the same as that contained in the calcium carbonate, viz. 14 grams.

In the second extraction, the lime and phosphoric acid found are very nearly or are practically exactly in the ratio of 3CaO : P$_2$O$_5$ so that apparently the salt dissolved is Ca$_3$P$_2$O$_8$.

In the third extraction, the amount of lime dissolved is nearly twice as much as that necessary to combine with the P$_2$O$_5$ found to form Ca$_3$P$_2$O$_8$, indicating that lime which has gone into solution is derived from calcium citrate, as well as from calcium phosphate.

In the fourth extraction, the amount of lime dissolved is nearly $6\frac{3}{4}$ times as much as that necessary to form Ca$_3$P$_2$O$_8$ with the P$_2$O$_5$ found. This appears to indicate that a larger proportion of the lime which has gone into solution has done so as calcium citrate than as calcium phosphate.

It will be seen also that the so-called "citrate soluble" phosphoric acid has fallen from 91 % to 84·4 % of the total phosphoric acid present.

Experiment (c). Extraction of 5 grams of an intimate mixture consisting of 50 % pure tricalcic phosphate and 50 % pure calcium carbonate with 500 c.c. of a 2 % solution of citric acid.

		gram CaO	gram P_2O_5
2·50 grams tricalcic phosphate containing 50·12 % CaO, 42·36 % P_2O_5 will contain		1·2530	1·0590
2·50 grams calcium carbonate containing 56·0 % CaO will contain		1·4000	—
5·00 grams mixture will contain		2·6530	1·0590

	grams CaO	grams P_2O_5	
100 grams of this mixture ∴ contains {	25·06	21·18	from the phosphate
	28·00	—	from the carbonate
	53·06	21·18	

Note that in this mixture there is an excess of 28 % of lime.

	Lime		Phosphoric acid			
	Grams found calculated to 100 grams mixture	Expressed in % of total lime present	Grams found calculated to 100 grams mixture	Expressed in % of total P_2O_5 present	Lime calculated from P_2O_5 found to form with it $Ca_3P_2O_8$	Difference between calculated lime and that actually found
1st extn, 30 min.	49·45	93·23	17·82	84·26	21·08	+28·37
2nd ,, 30 ,,	3·26	6·15	2·87	13·57	2·40	+ 0·86
3rd ,, 30 ,,	0·20	0·38	0·28	1·32	0·33	− 0·13
4th ,, 30 ,.	0·13	0·24	0·18	0·85	0·21	− 0·08
Total ...	53·04	100·00	21·15	100·00	24·02	+29·02
						53·04
Actually present	53·06	—	21·18	--	—	—

It will be noted that 93·2 % of the total lime and 84·3 % of the total phosphoric acid present have gone into solution in the first extraction. The lime extracted is 28·4 grams more than the amount necessary to form $Ca_3P_2O_8$ with the P_2O_5 found, an amount which is nearly the same as that contained in the calcium carbonate added. In the second extraction the lime found is considerably more than that which would be required to combine with the P_2O_5 found to form $Ca_3P_2O_8$, indicating that a considerable amount of lime has gone into solution as calcium citrate. In the third extraction the amount of lime found is just about the amount which would be necessary to produce dicalcic phosphate (·22) with the P_2O_5 found. The same remark applies to the amount of lime found in the fourth extraction, the lime and phosphoric acid found are in the proportions in which they exist in dicalcic phosphate.

It will also be noted that here again the so-called "citrate soluble" phosphoric acid has fallen from 91 % as in experiment (a) to 84·3 % of the total phosphoric acid present.

The extraction of mixtures of a higher calcium carbonate content was not tested, as it was considered that in practice few phosphates

would be submitted for the determination of "citrate soluble" phosphoric acid, which would contain more than 50 % calcium carbonate.

Before discussing further the results of these experiments on the action of the prescribed 2 % citric acid solution on tricalcic phosphate and mixtures of these with calcium carbonate, it will perhaps be well to give here, the results of investigations of the action of 2 % citric acid solution on the substances which were sold and purchased as tricalcic phosphate, but which chemical analysis showed to be mixtures of di- and tricalcic phosphates in varying amounts. For this purpose phosphates No. 1 and No. 2 were selected, the former containing 78 % tri- and 14 % dicalcic phosphate, and the latter 78 % di- and 14 % tricalcium phosphate.

The results of these two examinations will now be given.

Extraction of 5 grams of the phosphate of lime (No. 1) with 500 c.c. of a 2 % citric acid solution for 30 minutes.

This powder was shown to consist of:

Dicalcic phosphate $CaHPO_4$	...	...	14·188
Tricalcic phosphate $Ca_3P_2O_8$	...	...	78·271
Water of crystallisation	...	...	7·541
			100·000

containing lime (CaO) 48·26 % and phosphoric acid (P_2O_5) 43·26 %.

	Lime		Phosphoric acid		Lime	Difference
	Grams found calculated to 100 grams sample	Expressed in % of total lime present	Grams found calculated to 100 grams sample	Expressed in % of total P_2O_5 present	calculated from P_2O_5 found to form with it $Ca_3P_2O_8$	between calculated lime and that actually found
1st extn, 30 min.	39·91	82·58	36·16	83·64	42·78	−2·87
2nd ., 30 ,,	8·42	17·42	7·07	16·36	8 37	+0·05
Total ...	48·33	100·00	43·23	100·00	51·15	−2 82
					48·33	
Actually present	48·26	—	43·26	—	—	—

In the first extraction this salt appears to have gone into solution as a mixture of tri- and dicalcic phosphates, the tricalcic phosphate predominating, since the lime found is less than that necessary to combine with the P_2O_5 found to form $Ca_3P_2O_8$.

In the second extraction the phosphate appears to have gone into solution as tricalcic phosphate. There is a very minute excess of lime which probably indicates calcium citrate.

Extraction of 5 grams of the Calcii Phosphas B.P. (No. 2) with 500 c.c. of a 2 % citric acid solution for 30 minutes.

This powder was shown to consist of:

Dicalcic phosphate $CaHPO_4$	...	...	78·059
Tricalcic phosphate $Ca_3P_2O_8$	...	...	14·777
Water of crystallisation	...	...	...	7·164
			100·000

containing lime (CaO) 40·15 % and phosphoric acid (P_2O_5) 47·52 %.

	Lime		Phosphoric acid		Lime calculated from P_2O_5 found to form with it $Ca_3P_2O_8$	Difference between calculated lime and that actually found
	Grams found calculated to 100 grams samp'e	Expressed in % of total lime present	Grams found calculated to 100 grams sample	Expressed in % of total P_2O_5 present		
1st extn, 30 min.	33·18	83·14	39·46	83·00	46·68	− 13·50
2nd „ 30 „	6·73	16·86	8·08	17·00	9·56	− 2·83
Total ...	39·91	100·00	47·54	100·00	56·24	− 16·33
					39·91	
Actually present	40·15	—	47·52	—		

In the first extraction the phosphate experimented with appears to have gone into solution as a mixture of di- and tricalcium phosphate, the dicalcic phosphate predominating.

In the second extraction the lime and phosphoric acid found indicate a dicalcic phosphate, with very minute quantities of tricalcic phosphate.

When di-barium phosphate is dissolved in hydrochloric acid, barium chloride and free phosphoric acid are first produced in accordance with the equation $BaHPO_4 + 2HCl = BaCl_2 + H_3PO_4$. According to Newth[1] the addition of an alkali causes precipitation of the phosphate unchanged. The reaction proceeds in two stages—(1) the alkali added (say NaOH) reacts with the free H_3PO_4 forming sodium phosphate, and (2) the sodium of the sodium phosphate takes the chlorine from the barium chloride, and the barium of the barium chloride unites with the phosphoric acid of the sodium phosphate. These reactions are represented by the following equations:

$$BaCl_2 + H_3PO_4 + 2NaOH = BaCl_2 + Na_2HPO_4 + 2H_2O \quad \ldots\ldots\ldots\ldots(1),$$
$$BaCl_2 + 2Na_2HPO_4 + 2H_2O = BaHPO_4 + 2NaCl + 2H_2O \quad \ldots\ldots\ldots\ldots(2).$$
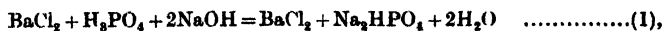
The solution of tricalcic phosphate in acid and its precipitation by an alkali has not been discussed in any text-book or paper that I have had access to, but the following reactions suggest themselves as such as would occur.

Solution in hydrochloric acid would be represented by the equation

[1] Newth, *Manual of Chemical Analysis*, p. 64.

$Ca_3P_2O_8 + 6HCl = 3CaCl_2 + 2H_3PO_4$. The addition of sodium hydrate would be represented thus:

$$3CaCl_2 + 2H_3PO_4 + 4NaOH = 3CaCl_2 + 2Na_2HPO_4 + 4H_2O \dots\dots\dots\dots(1),$$

and then $\quad^\bullet\quad 3CaCl_2 + 2Na_2HPO_4 + 4H_2O = Ca_3P_2O_8 + 4NaCl + 4H_2O + 2HCl \quad\dots\dots(2).$
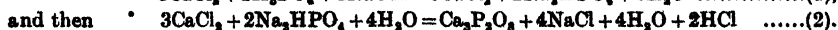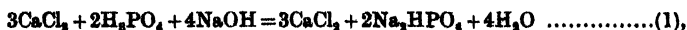
Were it possible to produce Na_3PO_4 in the reaction we would have:

$$3CaCl_2 + 2H_3PO_4 + 6NaOH = 3CaCl_2 + 2H_3PO_4 + 6H_2O \quad\dots\dots\dots\dots(1),$$

and subsequently

$$3CaCl_2 + 2Na_3PO_4 + 6H_2O = Ca_3P_2O_8 + 6NaCl + 6H_2O \quad\dots\dots\dots\dots(2),$$
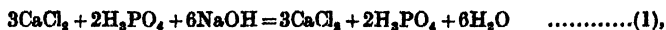
but as I know of no similar reaction in which Na_3PO_4 is produced but invariably Na_2HPO_4, no other conclusion seems possible but that free hydrochloric acid is produced which would react at once with the tricalcium phosphate present. In the case of ammonia being the precipitating alkali the following equations might represent what occurs:

$3CaCl_2 + 2H_3PO_4 + 6NH_4HO = (a) \quad 2(NH_4)_3PO_4 + 6H_2O$ (which is very improbable)

or $\qquad\qquad\qquad (b) \quad 2(NH_4)_2HPO_4 + 2NH_3 + 6H_2O \qquad\qquad$ (1),

$\qquad\qquad\qquad\qquad\qquad$ (or $2NH_4HO + 4H_2O$)
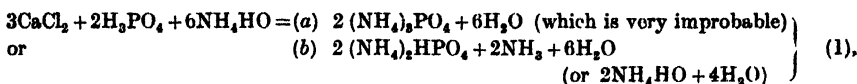
and then (a) and (b) yielding $\qquad Ca_3P_2O_8 + 6NH_4Cl + 6H_2O \quad\dots\dots\dots\dots\dots(2),$

or we might have with less ammonia:

$$3CaCl_2 + 2H_3PO_4 + 4NH_4HO = 2(NH_4)_2HPO_4 + 4H_2O + 3CaCl_2 \quad\dots\dots\dots(1),$$

and subsequently

$$2(NH_4)_2HPO_4 + 4H_2O + 3CaCl_2 = Ca_3P_2O_8 + 4NH_4Cl + 4H_2O + 2HCl \dots\dots(2),$$

if the latter alternative obtains, we would again have free hydrochloric acid, as is apparently the case when sodium hydrate is used, and which would immediately react with the tricalcic phosphate present

From analogy one might expect the primary action of citric acid on calcium phosphates to be similar to that of hydrochloric acid, and that calcium citrate and free phosphoric acid are produced at least primarily. Thus:

$$Ca_3P_2O_8 + 2H_3(C_6H_5O_7) = Ca_3(C_6H_5O_7)_2 + 2H_3PO_4$$
$$1 \quad : \quad 1\cdot0239$$
$$3CaHPO_4 + 2H_3(C_6H_5O_7) = Ca_3(C_6H_5O_7)_2 + 3H_3PO_4.$$
$$1 \quad : \quad 0\,931$$
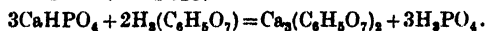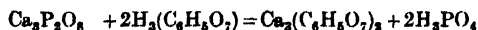
It will be noted that theoretically 5 grams of calcium phosphate would require about 5 grams citric acid for the above reaction whereas in the prescribed method of dissolving calcium phosphates in citric acid solution 10 grams of citric acid are prescribed.

A secondary reaction undoubtedly occurs since the filtrate from the reaction after 30 minutes contains both lime and phosphoric acid. The following equations are suggestions as to what may occur:

$$Ca_3(C_6H_5O_7)_2 + 2H_3PO_4 = 2H_3(C_6H_5O_7) + Ca_3P_2O_8$$
$$Ca_3(C_6H_5O_7)_2 + 3H_3PO_4 = 2H_3(C_6H_5O_7) + 3CaHPO_4$$
$$Ca_3(C_6H_5O_7)_2 + 6H_3PO_4 = 2H_3(C_6H_5O_7) + 3CaH_4P_2O_8.$$
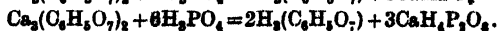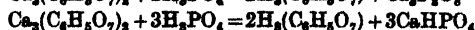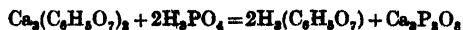
When calcium carbonate is dissolved in citric acid solution the addition of phosphoric acid causes no precipitate.

Calcium phosphates dissolve in citric acid solutions, the solution remaining clear. These solutions will then dissolve large quantities of calcium carbonate and still remain clear. On continuing the addition of calcium carbonate, a critical point is reached at which a faint cloudiness or precipitate appears. This is a mixture of calcium phosphate and calcium citrate, but immediately after the cloudiness has become apparent the solution becomes like a magma and calcium citrate is precipitated which contains only traces of phosphoric acid. Apparently then in a mixture of calcium phosphate and citrate lime salts precipitate calcium citrate. Again calcium citrate in aqueous solutions of calcium phosphate renders both lime and phosphoric acid soluble.

The following was tried:

A, Calcium phosphate + 75 c.c. water. B, Calcium phosphate (as in A), calcium citrate, 75 c.c. water. Both were allowed to stand overnight. On filtering aliquots of A and B it was found that filtrate A contained both lime and phosphoric acid while filtrate B contained about 10 times as much lime as filtrate A with only about $3\frac{1}{2}$ times as much phosphoric acid as in filtrate A.

SUMMARY.

The results of these investigations show:

1. The substances sold as "Phosphate of lime" and "Calcii Phosphas B.P." are not pure tricalcic phosphate but are mixtures of di- and tricalcic phosphates.

2. Sodium phosphate (Na_2HPO_4) added to ammoniacal calcium chloride and the resulting precipitate washed with water yields a mixture of di- and tricalcic phosphate and calcium hydrate.

3. Bone ash dissolved in hydrochloric acid and precipitated with ammonia, the precipitate being well washed, yields also a mixture of di- and tricalcium phosphate and calcium hydrate.

4. When three equivalents of lime (3CaO) are made to act on one equivalent of phosphoric acid (P_2O_5) and the resulting precipitate removed with little delay pure tricalcium phosphate is obtained.

5. When two equivalents of lime (2CaO) are made to act on one equivalent of phosphoric acid (P_2O_5) the product is not dicalcic phosphate but is a mixture of di- and tricalcium phosphate.

6. In the case of pure tricalcium phosphate, about 91 % of the total phosphoric acid is soluble in the prescribed 2 % citric acid solution

in 30 minutes when following the method adopted for the determination of "citrate soluble" phosphoric acid.

7. By the simple addition of calcium carbonate to a pure tricalcic phosphate, the "citrate solubility" of the phosphoric acid is reduced from 91 to 84 %.

8. The availability of the phosphoric acid as judged by the extraction with 2 % citric acid solution is inexact, since the availability of the phosphoric acid in pure tricalcium phosphate is reduced from 91 % to 84·5 % by the addition of 14 % of lime as carbonate, and to 84·3 % by the further addition of 14 % lime as carbonate.

9. The prescribed 2 % citric acid solution is more correctly a solvent for lime than for phosphoric acid, since broadly speaking, the whole of the excess of lime beyond that present as tricalcic phosphate goes into solution in the first 30 minutes' extraction.

10. Pure tricalcic phosphate is largely soluble in 2 % citric acid solution, as are also the so-called tricalcic phosphates produced by the addition of ammonia to acid solutions of tricalcic phosphate, or by the mixing of disodium hydrogen phosphate with ammoniacal calcium chloride. (These are mixtures of di- and tricalcium phosphate in varying amounts.)

11. Since tricalcic phosphate and dicalcic phosphate are both soluble in the prescribed 2 % citric acid solution the statement that dicalcic phosphate can be differentiated from tricalcic phosphate, by means of the selective action of this solvent, is untenable.

It follows that the manurial value of phosphates cannot be determined by a 2 % citric acid solvent in the method prescribed, and it therefore is a matter for consideration whether or not the further use of this method should be continued.

APPENDIX.

A paper by R. Warington[1], published in the *Journal of the Chemical Society*, 1873, has been quoted extensively by authors of text-books on chemistry as the authority on the decomposition of $Ca_3P_2O_8$ on boiling this with water, and the conclusion formed is that $3Ca_3P_2O_8 . CaOH_2O$ agrees best with the basic salt produced.

[1] R. Warington, "On the decomposition of Tricalcic Phosphate by Water," *Journal Chemical Society* (Entire Series), Vol. xxvi, p. 983.

$Ca_3P_2O_8$ was prepared by pouring pure $CaCl_2$ into a very dilute solution of Na_2HPO_4 plus 1 equivalent of ammonia. The alkaline phosphate was kept in excess during precipitation. The triphosphate was washed by decantation till no chlorine was found in the washings. All analyses were made unless the contrary is stated by dissolving an unweighed quantity of moist phosphate in hydrochloric acid, precipitating the lime with ammonium oxalate in acetic acid solution and afterwards the phosphoric acid with magnesia mixture.

The following figures are then given as representing the CaO and P_2O_5 in the phosphates prepared.

	(a)	(b)	(c)	(d)
CaO	54·37	54·78	54·95	54·93
P_2O_5 ...	45·63	45·22	45·05	45·07
	100·00	100·00	100·00	100·00

Warington has apparently assumed that because the ratio of lime to phosphoric acid approximates that of $3CaO : P_2O_5$, the compounds obtained were necessarily tricalcium phosphates, and the possibility of these products being mixtures of di- and tricalcic phosphate, or of these with free lime, appears to have been overlooked.

Let us examine for a moment the figures obtained by Warington.

(a) $54·37CaO$ with $45·63P_2O_5$. Now while theory requires $53·98CaO$ to be associated with $45·63P_2O_5$ to form tricalcic phosphate, $54·37CaO$ is found present. Therefore we have either

(1) tricalcic phosphate plus free lime, or

(2) tricalcic phosphate plus dicalcic phosphate plus free lime.

(2) will obtain if the amount of free lime is at all greater than that required for supposition (1).

(b) $54·78CaO$ with $45·22P_2O_5$. Now theoretically the $45·22P_2O_5$ present would require $53·05CaO$ to form $Ca_3P_2O_8$, whereas there is $54·78CaO$ present. Again therefore we have either a mixture of tricalcic phosphate and free lime or of di-, tricalcic phosphate and free lime.

(c) $54·95CaO$ with $45·05P_2O_5$. To produce $Ca_3P_2O_8$ from $45·05P_2O_5$ would require $53·30CaO$, and there is actually $54·95CaO$ present. Here also we have either (1) a mixture of tricalcic phosphate and lime, or (2) a mixture of di- and tricalcic phosphate and lime.

In the first case a mixture of $98·35\%$ tricalcic phosphate (containing $53·30CaO$ combined with $45·05P_2O_5$) with $1·65\%$ lime (CaO) would agree with Warington's figures found, viz. $54·95CaO$ and $45·05P_2O_5$. Now if the free lime actually present exceeds $1·65$ then di- and tricalcic phosphate must be present and their amounts can be calculated but

only when the figure for free lime present is assumed or is known. Assuming that $(CaO)_2H_2OP_2O_5 . 4H_2O$ is the dicalcic phosphate present and that the tricalcic phosphate is $Ca_3P_2O_8$ and the lime present as hydrate $CaOH_2O$ the following mixtures will contain $54·95CaO$ associated with $45·05P_2O_5$.

	(1)	(2)	(3)	(4)
Tricalcic phosphate ...	97·83	95·27	88·12	81·24
Dicalcic phosphate ...	—	2·12	8·04	13·73
Calcium hydrate ...	2·17	2·61	3·84	5·03
	100·00	100·00	100·00	100·00

and the ratio of lime to phosphoric acid would still be

$$54·95CaO : 45·05P_2O_5.$$

(d) $54·93CaO$ and $45·07P_2O_5$. To produce $Ca_3P_2O_8$, $45·07$ grams P_2O_5 would require $53·32CaO$, whereas $54·93CaO$ is found present.

The remarks under (c) hold here also.

Since tricalcic phosphate has apparently not been obtained, the conclusions drawn by Warington are not justified.

The formula $(3Ca_3P_2O_8 . CaOH_2O)$ for the resulting product of boiling the above phosphates with water is deduced not from an analysis of the product but again from the ratio of CaO to P_2O_5, the water of hydration or combination present appears to be more or less problematical.

However, assuming that the above formula approximates the composition of the resulting basic product it appears to be equally possible that this is still a mixture of di- and tricalcic phosphate with lime for $2Ca_3P_2O_8 .2CaHPO_4 . CaO$ or $2 (CaO)_3P_2O_5 . (CaO)_2P_2O_5 . CaOH_2O$ would agree equally well with the numbers found.

Further investigations on this matter are therefore desirable.

THE FIXATION OF NITROGEN IN FAECES.

By ERIC HANNAFORD RICHARDS.

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IN the course of a general study of the chemical changes taking place in the manure heap, a number of experiments were made with the object of determining the loss of nitrogen during the aerobic fermentation of urine, straw and faeces, both separately and in various combinations. In the very first experiment with faeces, however, instead of finding a loss of nitrogen, appreciable gains were recorded, and this opened the enquiry which forms the subject of the present paper. In all the experiments a current of washed air was drawn for several hours daily through the flasks containing the substance under examination, any ammonia volatilized being retained by N/10 sulphuric acid. At the end of the experiment the total nitrogen left in the fermented substance, added to any nitrogen recovered in the wash flasks, was compared with the original total nitrogen.

The method of preparing the faeces for experiment was as follows. A sample of about 500 grams is taken from a pailful of the droppings and passed through a mincing machine. A fairly homogeneous product is then obtained from which suitable portions can be readily weighed out as required. In order to avoid loss of moisture, the prepared sample is kept covered, except when actually taking sub-samples. In practice, with reasonable speed, no difficulty is experienced on this account.

A suitable amount of substance is weighed out in the Kjeldahl flask direct, the latter being held to the hook of the balance by a wire loop. In the case of horse faeces there is no difficulty in weighing out exactly 5 or 10 grams if so desired, but with bullock and cow faeces this is impossible, and the approximate amount required is dropped into the flask and weighed by differences. With cow faeces it is desirable to place a glass or paper tube in the neck of the flask when introducing the substance, thus avoiding the smearing of the neck and sides. The

tube is, of course, withdrawn when the sample is safely at the bottom of the flask. The following figures are typical of the degree of accuracy possible with this method of sampling:

Accuracy of Sampling.

	(a)	(b)	(c)
Weight of horse faeces taken (grams)...	6·324	6·094	6·820
Weight of nitrogen found (grams) ...	0·0200	0·0193	0·0215
Per cent. nitrogen on fresh sample ...	0·315	0·317	0·315

All estimations were made in triplicate and the mean result used in calculating the weight of nitrogen taken for each experiment.

In experiments of this type it is essential to know what differences are really significant. It was found that with the quantities usually taken, the total error of experiment amounted to about 2·5 % of the original total nitrogen. In the actual experiments no change in the nitrogen content under 5 % can be regarded as significant.

In the first experiment with horse droppings 20 c.c. of tap-water were added to some of the units of the series, in order to make the liquid surface, exposed to the air currents, correspond with that in some parallel experiments with urine. After 74 days at laboratory temperature (13° C.) no change of nitrogen content had occurred in 7 out of the 9 units, but in the remaining two, to both of which tap-water had been added, an *increase* of total nitrogen of 7 % was recorded. In this experiment no ammonia was volatilized and recovered in the acid traps. The small amounts of ammonia sometimes recovered in later experiments are included in the total nitrogen figures in the tables. Although the possibility of nitrogen fixation was recognised, these results were attributed to some experimental error, such as leakage of unwashed air into the apparatus, and no particular notice was taken of the discrepancies, beyond taking steps to avoid leakage in subsequent experiments. In any case fresh horse faeces did not seem at all a favourable medium for the growth of the better known nitrogen fixing organisms. Remy[1] has stated that farmyard manure is an unsuitable medium for *Azotobacter*. He found a loss of nitrogen in his experiments. The *Azotobacter* group are said to dislike a nitrogenous environment and, if they could have developed freely, would have fixed more nitrogen than the amounts actually found. On the other hand, the *Clostridia* are chiefly, if not exclusively, anaerobic and the conditions of the experiment were strongly aerobic. Nevertheless the possibility is not

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[1] *Centr. f. Bakt.* 2 Abt. Bd. XXII, 1909, p. 588.

wholly excluded, for the power to fix nitrogen to a limited extent is possessed by many organisms of the class which cause butyric acid fermentation in carbohydrate media.

Since these experiments were begun J. Hanzawa[1], working with mixed strains of *Azotobacter*, obtained greater fixation in mannite than with the same in pure culture, and notes a point of special interest in. the present connection, viz. that the humus of stable manure is capable of being utilized as a source of nitrogen fixation, while that of green manure is not. Presumably by humus he means the organic residue of rotted manure. The material used in the present experiments was always fresh from the animals. Hanzawa states that the presence of humus nitrogen or nitrates was almost without effect on fixation by *Azotobacter*; but if the nitrogen to carbon ratio exceeded 1 : 40 fixation was retarded.

More recently Tottingham[2], working with mixed cow and horse manure (faeces?), both with and without straw, has observed gains of nitrogen up to 5 % after four weeks' fermentation. He believes that the gain is due to the straw but was unable to detect *Azotobacter*.

A second series of experiments with horse faeces was set up and run for 119 days at laboratory temperature (15° C.). As before, the faeces containing only *natural* moisture showed no change of total nitrogen, but both the units to which tap-water was added gave an increase of total nitrogen that could not possibly be ascribed to experimental error. No attempt was made to collect the faeces aseptically, but to ensure that the normal soil organisms should be present originally, 1 c.c. of garden soil extract was added to one flask of each pair. The figures obtained in this experiment are given in the following table.

Horse faeces.

	Faeces alone	Faeces and tap-water	Faeces and soil extract	Faeces, tap-water and soil extract
Weight of substance taken (grams)...	5·032	5·028	5·028	5·028
Original total nitrogen (grams) ...	0·0147	0·0147	0·0147	0·0147
Final total nitrogen	0·0145	0·0188	0·0150	0·0205
Difference	− 0·0002	+ 0·0041	+0·0003	+ 0·0058
Per cent. of original total N.* ...	− 1·1	+27·9	+2·3	+39·4

* In the estimations of nitrogen the results were calculated to four figures but only three are given in the tables. The original four figures have been used in calculating the percentage difference.

[1] *Centr. f. Bakt.* 2 Abt. Bd. XLI, 1914, p. 573.
[2] *Journal Bio. Chem.* (Baltimore), XXIV, No. 3, 1916, p 221.

Having established the fact that nitrogen fixation actually occurred during aerobic fermentation of the horse faeces, the next step was to compare the behaviour of bullock and cow faeces under similar conditions. It is well known that the horse does not digest its food as thoroughly as the bullock. On the assumption that the nitrogen fixing organism derives its nutriment from the imperfectly digested carbohydrate in the fresh faeces, the amount of nitrogen fixed in bullock and horse dung should be in inverse relation to the digestive efficiency of the animals. The tap-water used in these experiments contained much calcium carbonate which apparently favoured nitrogen fixation, for it proved more effective than distilled water. To test this point one of each pair in the next series of experiments was made up with distilled instead of tap-water. The results of 134 days' fermentation at laboratory temperature are set out below.

Comparative fixation of Nitrogen in Horse and Bullock faeces.

	Horse faeces and tap water	Horse faeces and distilled water	Bullock faeces and tap water	Bullock faeces and distilled water
Weight of substance taken (grams)	5·001	5 000	10 008	9 897
Original total nitrogen (grams) ...	0 0132	0·0132	0·0258	0·0255
Final total nitrogen	0 0191	0 0164	0 0285	0 0269
Difference	+ 0 0059	+ 0·0032	+ 0 0027	+0 0014
Per cent. of original total N ...	+45·3	+24 8	+ 10 4	+5 2

The figures suggest that the view just put forward is sound, as the bullock droppings fixed less than a quarter the amount of nitrogen gained by the corresponding samples of horse faeces. Distilled water, again, had a marked depressing action compared with tap-water.

It is obvious that the diet of the animals must influence such results considerably. In this case the horses were receiving the ration of hay and oats usual for work on the farm, and the bullocks were on grass with about 5 lbs. of mixed cotton and linseed cake per head per day in addition.

The effect of a purely grass diet on the nitrogen fixing power of horse faeces was tested in two experiments with the following results:

Horse faeces on grass.

	Sample No. 1	Sample No. 2
Weight of substance taken (grams) ...	7·430	6·521
Original total nitrogen (grams) ...	0·0180	0·0158
Final total nitrogen	0 0197	0·0170
Difference	+0·0017	+0·0012
Per cent. of original total N	+9 4	+7·6

It appears that a grass diet without any corn ration reduces the value of horse faeces regarded as a nitrogen fixing medium to about the level of grass and cake fed bullock droppings. Bullocks and cows on grass alone seem to produce nothing in their excrement which can function as food for nitrogen fixers—under the conditions of these experiments at any rate—but the relatively poor digestion of the horse leaves sufficient carbohydrate unassimilated to show an amount of fixation well beyond the experimental error.

In the next experiment horse, bullock, and cow faeces, all, however, on different diets, were run in parallel for 112 days. To each flask was added 0·1 gram calcium carbonate, 0·1 gram of garden soil, and 25 c.c. tap-water. Each variety of dung was tested in triplicate and the mean results are given below.

Comparative fixation of Nitrogen in Horse, Bullock and Cow faeces.

	Horse faeces	Bullock faeces	Cow faeces
Weight of substance taken (grams) ...	5·000	11·941	7·598
Original total nitrogen (grams) ...	0·0165	0·0523	0·0230
Final total nitrogen	0·0208	0·0513	0·0217
Difference	+ 0·0043	– 0·0010	– 0·0013
Per cent. of original total N.	+25·8	– 1·9	– 5·7
Mgms. of nitrogen fixed per gram of dry matter in faeces	+ 3·0	– 0·1	– 0·6

The sample of horse faeces was evidently not as effective as the previous one and neither the bullocks nor cows were getting any cake when these samples were taken (August, 1915). In this experiment the bullock faeces did not fix even the small amount of nitrogen found in the last experiment. The cow dung actually lost a little nitrogen, thus falling into its natural position on the hypothesis that fixation is a function of undigested residues from concentrated foods.

To complete this part of the work it was necessary to examine the droppings of a cow fed on a cake diet. By the kindness of Mr C. F. Benson, manager of the Hon. Rupert Guinness' farm at Woking Park, a suitable sample was obtained from an animal specially fed for the purpose of this experiment which was run for 28 days with the results set out below.

Even when the animal was fed on an abnormally rich diet cow faeces fixed practically no nitrogen, but it is significant that both the duplicate experiments show an increase close to the experimental error,

while the grass fed cow dungs used in the preceding experiment all gave negative results.

Fixation of Nitrogen in Cow faeces (cake fed).

	Faeces, tap-water and chalk		Faeces, tap-water, chalk and soil	
	(a)	(b)	(a)	(b)
Weight of substance taken (grams)...	5·076	7·466	5·335	5·577
Original total nitrogen (grams)	0·0159	0·0234	0·0184	0·0191
Final total nitrogen ...	0·0149	0·0224	0·0194	0·0194
Difference	−0·0010	−0·0010	+0·0010	+0·0003
Per cent. of original total N.	−6·5	−4·3	+6·1	+1·5

Effect of diet on Nitrogen fixation.
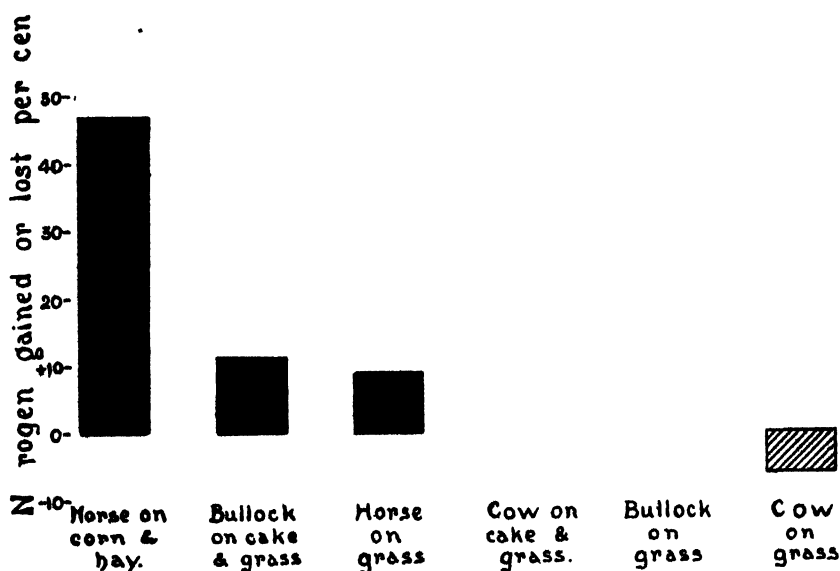
Fig. 1.

Fig. 1 shows diagrammatically the effect of diet on the nitrogen fixing power of faeces from the horse, bullock and cow.

Before turning to the bacteriological aspect of these nitrogen changes, there is one other point which may or may not have any direct connection with the fixation of atmospheric nitrogen that is undoubtedly associated with normal horse faeces under conditions not very different from those operative in the field.

One of the first outdoor experiments with manure, forming part of the general enquiry still in progress, showed that ordinary stable (horse) manure when stored in loosely made heaps for three months in the open, lost far less total nitrogen than similar heaps of cow dung. Duplicate heaps of both manures gave results in very close agreement. It is not possible to go fully into the matter here and only those points of interest in the present connection are touched on.

The proportion of ammoniacal (i.e. the most easily lost) nitrogen to total nitrogen was actually higher in the horse manure originally, yet these heaps lost only 10 % of their nitrogen while the cow dung lost 24 %. Two factors believed to favour loss of nitrogen, viz. aeration and temperature, were both predominant in the horse manure. After storage both kinds of dung had lost practically all their ammonia, but while the cow dung had also lost a considerable amount of more complex nitrogen, the horse dung showed an increase of 10 % in this form.

The nitrogen changes in the two kinds of manure are most easily seen in the diagram below (Fig. 2).
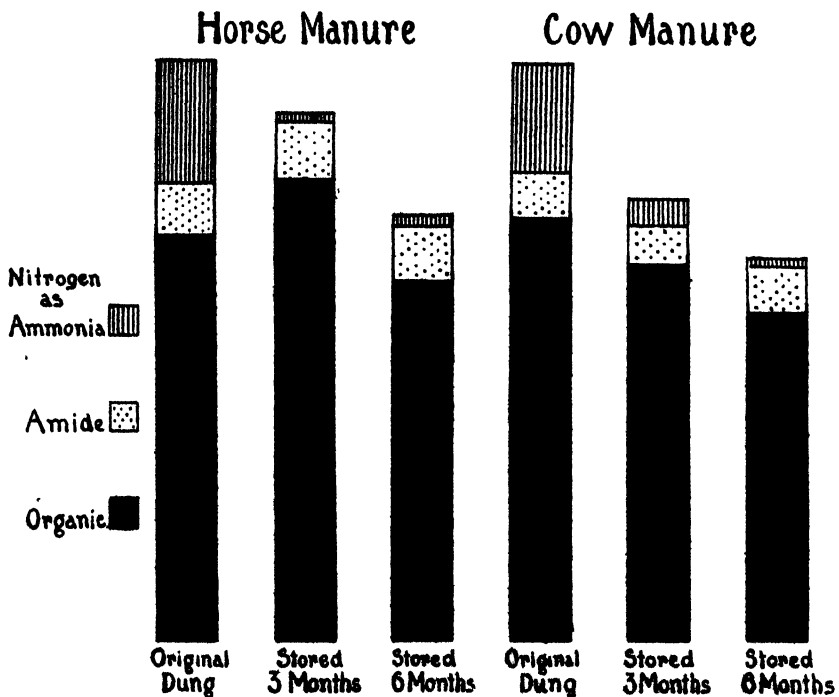
Fig. 2. Changes in manure during storage.

From what source does this newly formed protein get its nitrogen? The most obvious answer is from the ammoniacal nitrogen derived from the urine. But there is another possibility. Atmospheric nitrogen may have been fixed by the same process at work in the laboratory experiments. Further there is some evidence that the substances available as food for nitrogen fixing organisms can also nourish those which convert ammonia into more complex forms.

Both these actions may have gone on in the heaps of horse manure during the first three months of storage, while the special food material was available. In the second period of three months, after the exhaustion of this special food, there is a considerable loss of organic nitrogen. Cow manure on the other hand loses this form of nitrogen from the first.

The earlier experiments described above were all run for periods of at least four months with the idea of giving the maximum opportunity for nitrogen changes to occur, but it was not known how long fixation took: whether action was complete in the first brief period or spread uniformly over the whole interval. A series of flasks was therefore set up each containing 5 grams of fresh horse faeces, 0·1 gram calcium carbonate, 0·1 gram of garden soil and 25 c.c. tap-water. Washed air was drawn through them for about 3 hours daily as usual. At intervals a flask was removed and the nitrogen determined. In most of the previous experiments it was noticed that a distinct brown film formed on the surface of the liquor after two or three weeks. Towards the end of the present experiment the film was so tough that it required vigorous shaking of the flask to break it. The nature of this scum will be referred to later; it is probably physical rather than biological in origin. The question then arose, could the film cut off the supply of air to the bulk of the fermenting substance and so limit the nitrogen fixation? The inlet tubes of all the flasks used in these experiments delivered their air close to, but not touching, the fermenting substance. The air did not bubble through the solid or liquid. For the last eight weeks of this experiment the inlet tubes of two of the flasks were pushed down through the film so that the bubbling air should break it up. As the figures in the following table show, the effect of this bubbling was to increase to some extent the amount of nitrogen fixed.

These figures indicate that the fixation of nitrogen was complete in the first 28 days and that the maximum activity probably occurred in the third week of the experiment. From the 28th to the 112th day the amount of nitrogen fixed was very uniform in all the flasks: the greatest

difference was only 2·6 %, or just about the experimental error of the method.

Time taken for Nitrogen fixation.

Days	Weight of nitrogen, grams	Per cent. increase	Remarks
0	0·0165	—	—
14	0·0176	6·1	—
28	0·0190	15·1	—
42	0·0191	15·5	Film beginning to form.
56	0·0194	17·2	Film increased.
84	0·0190	15·1	Film very strong.
84*	0·0196	18·5	Film broken up.
112	0·0190	15·1	Film very strong.
112*	0·0208	25·8	Film broken up.

* Air bubbled through.

As will be seen subsequently the amount of nitrogen, 2·5 mgms., fixed in 28 days corresponds fairly well with the 2·2 mgms. fixed by a mixed culture of *Azotobacter* and *B. lactis aerogenes* acting on 0·5 gram of starch for 25 days at 20° C.

An attempt was made to cultivate the nitrogen fixing organisms which had operated so energetically in the first compatative experiment with horse and bullock faeces (p. 302) by inoculation from one tap-water unit of each type. This was not done until the close of the experiment, i.e. 134 days from the start, as the information afforded by the last of the horse dung experiments was not then available. Repeated trials with 2 % mannite-mineral salt solution gave negative results. The inoculations should have been made from the 14th to the 28th day during the period of active fixation. Examination of material from the fermentation flasks revealed nothing like *Azotobacter* or *Clostridium*. Bacterial life of any kind appeared to be in abeyance but time did not permit of any thorough examination of the types present.

The brown film referred to previously seemed to be a mechanically formed skin analogous to that forming on heated milk rather than a biological scum or zoogloea. It puckered in just the same way as milk and had a rather silky surface. There were no bubbles.

Concurrently with the chemical examination of the final series of horse dung experiments (p. 306), inoculations were made into both mannite and dextrose media. To each 50 c.c. of 2 % solution 1 gram of CaCO₃ was added and the flasks incubated at 25° C. The first inoculation from the 14-day flask gave considerable growth in mannite and still more in dextrose. The most conspicuous organism in both cases

was a short rod bacillus enclosed in a mucilaginous capsule. No *Azotobacter* or *Clostridium* could be detected, though in subsequent inocula-tions from the faeces in the later fermentation flasks, one or two *Azotobacter* forms were found. After three or four cultivations in dextrose the capsuled organism was obtained in practically pure culture. Young cultures of the first generation fixed 3 to 4 mgms. of nitrogen per gram of dextrose producing considerable butyric acid. None of these remarks refer to pure cultures, as no plates were poured, so that it cannot be said that the fixation was due to a single organism.

By plating out on dextrose-chalk-agar from raw cultures in mannite inoculated with the garden soil used in all the experiments two organ-isms were isolated, (1) a variety of *Azotobacter*, (2) *B. lactis aerogenes* (?).

They had the following morphological and cultural characters: (1) *Azotobacter*. In cultures 48 hours old; cells oval $2 \times 4\mu$, motile; colonies on dextrose-chalk-agar are white at first, then yellow with raised centre and finally dark brown. In 14 days the colonies are lobed and may be as large as 10 mm. diameter. (2) *B. lactis aerogenes* (?). In cultures 48 hours old; cell rods $1.5—2.0\mu$ long, non-motile; colonies on dextrose-chalk-agar are transparent, moist; capsules $4\mu \times 2\mu$. Ferments dextrose, lactose, saccharose; gives Vosges-Proskauer re-action; gram negative.

The mixed culture was found to fix considerably more nitrogen when grown in a 2 % starch-phosphate medium than in corresponding dextrose medium.

Mgms. of Nitrogen fixed per gram of carbohydrate in solution.

		Mixed culture		*Azotobacter* alone
		Dextrose	Starch	Dextrose
14 days at 26° C.	...	1·97	3·54	1·84
14 „ 35° C.	...	3·32	4·39	—
25 „ 20° C.	...	—	4·39	1·49

As it is known that *Azotobacter* uses its carbohydrate more economic-ally at low concentrations, the gradual action of the enzymes of *B. lactis aerogenes* on the starch may account for the better fixation of nitrogen given by the mixed culture in that medium.

Dr H. B. Hutchinson was good enough to isolate the capsuled organism from a dextrose-agar plate and examine the pure culture. He was of the opinion that it was closely related to, or identical with, *B. lactis aerogenes*, and that the fixation of nitrogen in faeces may be

due to a combination with *Azotobacter*. Beijerinck and Van Delden[1] found that certain species of *Azotobacter chroococcum* in combination with other bacteria were able to fix nitrogen more readily than in pure culture. One of the organisms tested symbiotically by these workers was *B. lactis aerogenes*. Beijerinck, however, could only fix about 1 mgm. of nitrogen per gram of dextrose with his combinations of *A. chroococcum + B. lactis aerogenes*. The crude cultures from the horse faeces were three or four times as active, but this may be due to the fact that calcium carbonate was added to these, while Beijerinck apparently used none.

By the kindness of Professor A. Harden the strain of *B. lactis aerogenes* at the Lister Institute was compared with the pure culture of the capsuled organisms. They were found to be identical as far as tested.

Samples of horse faeces, treated in different ways, were incubated in duplicate *without air current*, at 18° C. for 14 days. 1 c.c. of dextrose culture was used for the inoculations.

Inoculation of Horse faeces. Series 1.

	Raw faeces +soil	Raw faeces +mixed culture	Sterile faeces +mixed culture	Sterile faeces + Azotobacter
Original total nitrogen (grams) ...	0·0260	0 0235	0·0197	0·0243
Final total nitrogen (grams)...	0·0292	0·0256	0·0192	0·0227
Difference ...	+ 0·0032	+0·0021	-0·0005	-0·0016
Per cent. of original total N. ...	+13·1	+9·2	-2·6	-6·6

Another sample of horse faeces incubated in a similar way for 30 days gave the following figures:

Inoculation of Horse faeces. Series 2.

	Raw faeces alone	Raw faeces +soil	Raw faeces +Azotobacter	Sterile faeces alone	Sterile faeces +soil
Original total nitrogen (grams) ...	0·0150	0·0150	0·0150	0·0154	0·0154
Final total nitrogen (grams) ...	0·0157	0·0161	0·0157	0·0160	0·0180
Difference ...	+0·0007	+0·0011	+0·0007	+0·0006	+0·0026
Per cent. of original total N. ...	+4·9	+7·5	+4·4	+3·8	+17·5

Raw faeces inoculated both with soil and with the mixed culture, all fixed small amounts of nitrogen, but after sterilization in the autoclave at 120° C., the only samples showing significant fixation were those inoculated with soil; these gave the greatest increase found in

[1] *Centr. f. Bakt.* 2 Abt. Bd. IX, 1902, No. 1, p. 3.

any experiment of the two series. It seems probable that the nitrogen fixation is only the last link in a chain of biological changes brought about by organisms normally present in the faeces or soil.

Since *Azotobacter* alone is unable to fix any appreciable amount of nitrogen in faeces, while in combination with *B. lactis aerogenes* it will fix three times as much nitrogen as a pure culture of *Azotobacter*, both grown in dextrose, and further, inoculation of raw faeces with the mixed organisms does fix a considerable amount of nitrogen, it may be concluded that these two organisms play some part in the changes observed in these experiments.

Has the nitrogen fixation noticed in these experiments any practical significance? The direct effect is probably small. At most it means that when a soil is manured with horse dung there is a possible addition of atmospheric nitrogen which may amount under the most favourable conditions to 30 % of the nitrogen in the complete manure. The quantity of stable manure applied to arable soil is small and is steadily diminishing as the motor replaces the horse for road traction. The amount of nitrogen that could possibly be fixed in this way is practically negligible. Any value seems rather to lie in the evidence that appreciable nitrogen fixation can occur under conditions generally held to be unfavourable for this form of bacterial activity. There are industrial by-products containing actual or potential food for organisms of the types working in horse faeces. Obviously many difficulties must be overcome before any use can be made of waste material in the way indicated, but it is along these lines that any attempts to increase the quantity of atmospheric nitrogen normally added to the soil should have some chance of success.

CONCLUSIONS.

1. Horse faeces contain material capable of fixing nitrogen when fermented aerobically in presence of sufficient moisture and calcium carbonate.

2. This fixation is a function of the diet, for when horses are fed on grass alone, instead of corn and hay, the amount of nitrogen fixed is much reduced.

3. Under the most favourable conditions 1 gram of dry matter in the faeces will fix 4 mgms. of nitrogen.

4. Bullock faeces will also fix nitrogen but to a much smaller extent than horse faeces. This is also a function of the diet as it only

occurs when the animals are fed with cake; on grass alone no nitrogen is fixed.

5. The organisms concerned in the fixation of nitrogen are present in garden soil.

6. Evidence is adduced to show that fixation is brought about by a mixed culture of *Azotobacter* and *B. lactis aerogenes*. Of these the latter is normally present in faeces; *Azotobacter* is not, but readily infects faeces. Both organisms are present in the soil used and will fix nitrogen in raw faeces but not in sterile faeces.

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THE SHRINKAGE OF SOILS.

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THE fact that all soils when compacted in a moist condition show a marked decrease in volume on drying is well known. The magnitude of the decrease observed varies in the case of different soil types, being greatest as a rule in the case of clay soils and least in those of open sandy texture. The effect is seen in practice in the cracking of the ground surface which occurs during prolonged spells of dry weather, especially in regions in which the prevalent soil type is close grained and heavy.

The earliest recorded attempts to study and to give numerical expression to this property were made by Schubler, in the course of his work on Soil Physics during the early part of the nineteenth century, who measured the volume contraction exhibited on drying by a number of soils and soil constituents. According to Warington, the actual cubical contraction observed in the case of a number of arable soils ranged from 12 to 15 per cent. of the original volume.

Until the year 1908 the question does not appear to have been further investigated quantitatively, when at the instance, and under the direction of F. Watts, investigations were undertaken in the West Indies with a view, primarily, to ascertaining whether any connection could be established between the magnitude of the effect observed in the case of certain soils and the condition of crops growing thereon, notably cacao.

The results achieved were summarised in a paper by G. G. Auchinleck published in the *West Indian Bulletin*, vol. XII, pp. 50–69, in which also the methods adopted were described. Briefly these consisted in the addition of water to air-dry soil accompanied by thorough kneading of the sample until the mixture of soil and water had attained its maximum degree of plasticity, the standard assumed being that the

soil can readily be moulded but at the same time does not adhere unduly to the hands or instruments used in the course of the preparation of the sample. Methods of standardising plasticity in the case of certain soils were described, consisting in the addition of measured quantities of water to the soil accompanied by kneading, as in the case of the titration of flour to ascertain the quantity of water required to prepare a standard dough, and also by means of the determination of the weight required to crush a block of soil when at the correct consistency.

The method of measurement consisted in preparing bricks of the kneaded soil of standard dimensions, the insertion therein of two marks at a known distance apart and the measurement of the distance between the marks after the bricks had dried. The difference between the original and the final measurement was expressed as a percentage of the original distance between the two marks and gives the coefficient of linear shrinkage in the case of the sample under examination.

Comparison of the results of such measurements with the condition of growing cacao on a number of soils in Dominica and St Lucia showed that over a range of 34 examples a very fairly close correlation could be observed, and that under the conditions of climate prevailing in these islands, when the shrinkage of the surface soil exceeded 10 per cent. and that of the subsoil exceeded 12 per cent. the conditions were unfavourable to cacao production.

In relation to the actual magnitude of the effect observed, over a large range of soil samples from different parts of the West Indies, the linear shrinkage has been found to vary from 2·0 per cent. in the case of a very open sandy soil to 16 per cent. in the case of an exceptionally heavy clay soil.

In a note appended to the paper in question the probable relationship between gel formation in the case of the soil colloids and the shrinkage observed, was briefly discussed by the present writer and attention directed to the approximate interdependence existing between the magnitude of the shrinkage observed in any case and the proportion of particles less than ·01 mm. in diameter present in the sample, the ratio $\dfrac{\text{percentage of particles less than ·01 mm.}}{\text{per cent. shrinkage observed}}$ exhibiting a rough degree of constancy.

Since the publication of the original paper the method has been employed fairly extensively by experiment station workers in the West Indies as a simple and convenient means of gaining a certain degree of information concerning the physical character of soils and

of studying in detail departures from the prevalent soil type over limited areas.

Recently the subject has been further investigated in relation to certain West Indian soil types in the writer's laboratory, and the results of these investigations are given below.

These investigations fall naturally into two parts; in the first the relationship existing between shrinkage and water loss is considered; in the second considerations affecting the proportionality existing between the observed shrinkage and the content of colloidal clay are dealt with.

PART I.

THE RELATIONSHIP BETWEEN CONTRACTION AND WATER LOSS.

The method of attacking the problem consisted in studying in detail the relationship existing between water losses and the corresponding linear contraction on blocks of soil from 20 to 25 c.c. in volume, measuring in each case incremental water losses and the corresponding increments of linear contraction.

EXPERIMENTAL.

Description of the apparatus employed[1].

The apparatus adopted took the form described below:

It comprised a small rectangular cage of brass wire gauze 1·6 mm mesh, and of the following dimensions, 8 cms.× 2·5 cms. ⁄ 2·5 cms., this cage was provided with a slit 6 mm. in width running along the centre of the upper surface parallel with the length. One end of the cage was capable of being opened and closed at will so as to permit the insertion therein of the block of soil under observation. The dimensions of the cage and the blocks of soil were such that the blocks fitted snugly inside the cage.

The cage was supported in a frame of stout copper wire, provided with a loop for suspending it. To the horizontal bar of the frame a scale graduated in millimetres was fixed, the arrangement being such that when the cage was placed in the frame the scale lay immediately above the slit in the upper surface of the cage previously referred to.

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[1] For certain features in the apparatus the writer is indebted to the paper by B. A. Keen on "The Evaporation of Water from Soil," This *Journal*, **6**, 456.

The arrangement of the cage and support is shown in Fig. 1. The cage and support were suspended by means of a light vertical metal rod from one end of the beam of a balance placed on a table above, a hole of suitable size being cut in the bottom of the balance case and of the table to permit the passage of the rod.

The cage and support were enclosed inside an ordinary bell jar standing on a thick ground glass sole, the light vertical suspending rod passing through the neck of the bell jar; the neck of the bell jar was closed by two microscope slides in which semicircular nicks had been filed, each of them being of a depth equal to half the diameter of the suspending wire, thus enabling the two slips to be closed edge to edge, the suspending rod passing through the small opening in the centre. Two other slips of glass were placed so as to cover the juncture of the
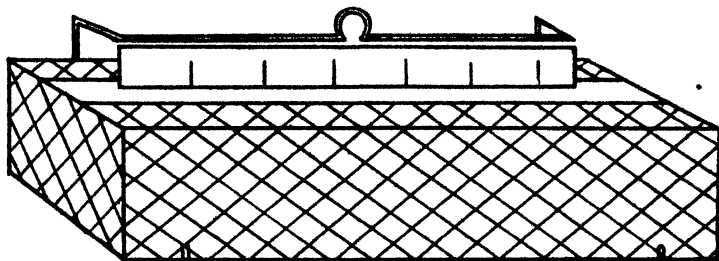
Fig. 1.

first two. The top and bottom edges of the bell jar were ground and received an ample application of grease so as to make the system tight. Within the bell jar a shallow glass dish was placed containing concentrated sulphuric acid so that a uniformly dry atmosphere was maintained within the system.

The tare weight of the cage and support were determined by counterbalancing them with weights on the right hand scale pan of the balance, subsequently the block of soil under observation was introduced and its weight also determined. In making a weighing the slips covering the neck of the bell jar were separated by a distance sufficient to allow the suspending rod to hang freely. The shrinkage was determined by inserting in the upper surface of the block of soil two glass threads which had been drawn off to a fine point and coloured red with red ink. These were placed at a suitable distance apart in the block, generally about 5 centimetres, and projected through the slit in the cage; they were of such length that their points were immediately opposite to and

almost in contact with the scale on the supporting frame. The reading of each point against the scale was made by means of a telescope. It was found that in this way the readings could be made to ·01 cm. The method followed in carrying out an experiment was to take readings of the two points on the scale and of the weight of the block at half hourly intervals until shrinkage had ceased, after which the block was dried to constant weight in the hot air oven at 110° C.[1]

In preparing the blocks of soil for a series of readings, the fine earth sample was employed, that is to say all particles having a diameter larger than 1 millimetre were removed by sifting. The amount of water requiring to be added to each sample under examination per 100 grms. of air-dry soil in order to produce maximum plasticity having been previously determined, the calculated amount of water was added in small quantities at a time, to a known weight of soil accompanied by thorough kneading, and the well-kneaded soil then moulded into a brick of the proper size by means of a wooden mould. The mould in question consisted of two wooden uprights fixed to a base at the required distance apart. In making a block the mould was lined with thin paper, the mould then filled with soil, the well-kneaded soil being pressed down tightly in the mould by means of a steel spatula; when sufficient soil had been transferred to the mould the paper lining was removed carrying the block with it; the ends of the block were trimmed by means of a sharp knife so as to reduce it to the proper length, the block inserted in the cage, the end of the cage closed, the two glass pointers inserted into the block, the cage placed in the support, the cage and support suspended from the metal rod in the drying chamber, and the series of observations commenced.

The plan of drying the blocks in a wire cage was adopted on account of difficulties experienced in the earlier stages of the work owing to uneven evaporation from different sides of the blocks, resulting in a tendency on the part of the blocks to buckle upwards, thus vitiating the observations of the shrinkage. The endeavour was first made to circumvent the difficulty by allowing the blocks to dry on a suspended wire gauze tray but this was not entirely satisfactory; the plan finally adopted of allowing the blocks to dry in a wire gauze cage was found to give very satisfactory results.

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[1] In the event of it not being possible to complete a series of readings in one day it was found that by removing the dish containing the sulphuric acid the readings could be interrupted over night and resumed the next morning without seriously disturbing their continuity.

The general arrangement of the apparatus is shown in Fig. 2. For the purpose of the investigation five typical soil samples were
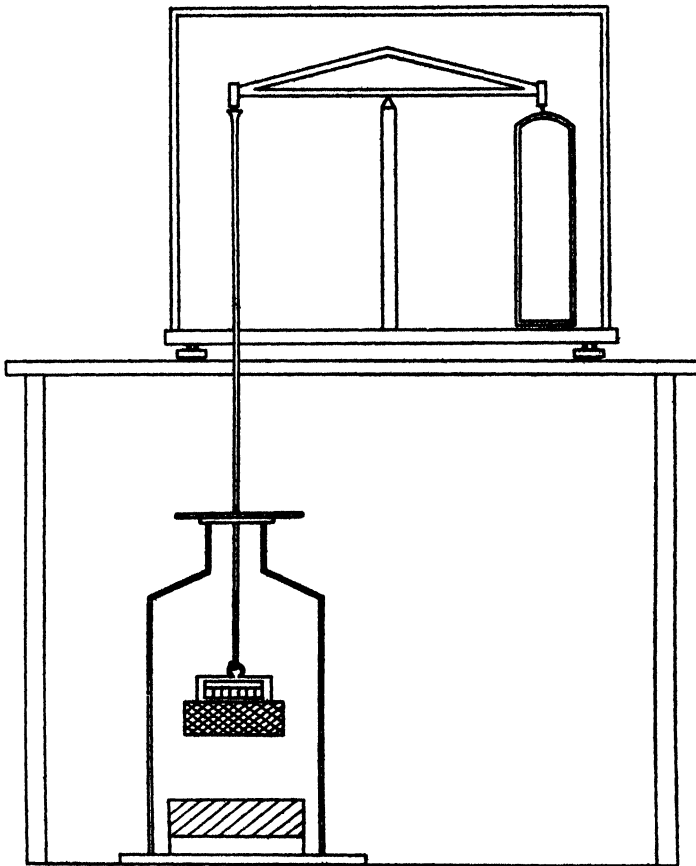
Fig. 2.

selected, taken from cultivated lands in different parts of the Leeward Islands Colony; they are described below:

    A. A heavy slightly calcareous clay loam.

    B. A heavy non-calcareous loam.

    C. A medium non-calcareous loam.

    D. A moderately light non-calcareous loam.

    E. A light non-calcareous loam.

The origins of the above soils are respectively as follows: A and B are from Antigua, C is from Nevis, D and E are from Montserrat.

The volume of water requiring to be added to 100 grms. of air-dry soil to produce maximum plasticity together with the mean total linear shrinkage expressed as a percentage in each case is given below.

	Volume of water required to produce maximum plasticity. C.c. per 100 grms. air-dry soil	Mean linear shrinkage per cent.
A.	37·0 c.c.	13·0 por cent.
B.	36·0 ,,	12·1 ,,
C.	26·0 ,,	8·8 ,,
D.	24·0 ,,	6·0 ,,
E	19·0 ,,	2·9 ,,

The physical composition of each of the soils in question as determined by Osborne's beaker method are given below:

Grade	Diameter of particles mm.	A	B	C	D	E
Stones	5 and over	—	3·6	3·8	7·4	3·0
Coarse Gravel ...	5 to 2	3·3	2·6	6·6	1·7	5·0
Gravel	2 to 1	3·6	5·6	9·1	3·5	7·6
Coarse Sand ...	1 to 0·5 ...	4·5	5·0	6·3	4·0	6·4
Medium Sand ...	0·5 to 0·25 ...	9·2	9·2	15·5	15·6	17·0
Fine Sand	0·25 to 0·1 ...	4·7	2·3	9·5	2·5	12·4
Very Fine Sand ...	0·1 to 0·05 ...	4·3	3·7	4·4	6·2	6·5
Silt	0·05 to 0·01 ...	7·4	7·8	4·7	9·9	11·0
Fine Silt	0·01 to 0·005 ...	47·9	44·1	28·7	37·7	25·6
Clay	less than 0·005	7·4	9·4	7·9	9·1	2·6
Organic matter and combined water	... ...	10·2	6·7	4·7	2·4	2·9
Total ...		102·5	100·0	101·2	100·0	100·0

An examination of the above figures will show that although a rough proportionality exists between the shrinkage observed and the content of particles of the fine silt and clay order of magnitude the proportionality is by no means exact, and in the case of the samples C and D the physical analysis would lead one to expect that a slightly greater shrinkage would be found to occur in the case of D than that of C while in fact the reverse is actually the case.

In the case of the various experiments the series of trials were in the majority of instances repeated a considerable number of times and the results obtained represent the mean of a number of independent determinations. It was found that this was the best plan to obtain readings which would give smooth curves, as, in individual instances, tendencies to depart from the smooth form were usually observed. These irregularities most usually tended to occur at the commencement

of a series of readings and were the result of the increased tendency of the blocks to stick to the supporting cage in the wet stage. This objection was partly surmounted by rubbing the interior surface of the cage with graphite but could not be completely got over. By repeating the experiments a number of times the irregularities could however be readily smoothed out.

The results are given in the accompanying table; they are expressed in terms of the water per cent. on the weight of the wet brick and in the corresponding linear contraction per cent. The results are also displayed graphically in Diagram 3 (p. 320).

A		B		C		D		E	
Water loss per cent on wet brick	Shrink-age per cent	Water loss per cent on wet brick	Shrink age per cent	Water loss per cent on wet brick	Shrink-age per cent.	Water loss per cent on wet brick	Shrink age per cent	Water loss per cent on wet brick	Shrink age per cent
2·0	1·55	2·0	1·55	2·0	1·55	2·0	1·55	1·0	·65
4·0	3·1	4·0	3·1	4·0	3·1	4·0	2·90	2·0	1·28
6·0	4·65	6·0	4·65	6·0	4·65	6·0	4·20	3·0	1·48
8·0	6·15	8·0	6·15	8·0	6·0	8·0	4·9	4·0	2·00
10·0	7·8	10·0	7·7	10·0	7·3	10·0	5·5	5·0	2·24
12·0	9·1	12·0	8·9	12·0	8·2	12·0	5·8	6·0	2·50
14·0	10·30	14·0	10·0	13·0	8·6	13·2	6·0	7·0	2·75
16·0	11·3	16·0	11·1	14·0	8·7	—	—	7·8	2·9
18·0	12·17	18·0	11·8	15·0	8·8	—	—	—	—
20·0	12·80	19·0	12·1	—	—	—	—	—	—
21·5	13·0	—	—	—	—	—	—	—	—

The character of the results is striking and shows that in the earlier stages of the process of evaporation and contraction the per cent. water lost and the corresponding per cent. linear contraction is the same in all cases, and takes the form of a straight line; as evaporation proceeds however the straight line form is departed from and the curves evince an upward tendency; the point of departure from the straight line form varies in the case of the different soils occurring at a point which is progressively nearer to the point of origin of the curve as the total observed shrinkage diminishes.

The reason underlying these occurrences appears fairly obvious when the mechanism of the process of shrinkage is considered. The effect in question is attributable to the action of soil colloids, which when soil is moistened form a gel; successive additions of water to dry soil result in the more and more complete transition to the gel form, until when the point of maximum plasticity is reached the gel is in equilibrium. At the point of maximum plasticity it appears probable

from the above result that the whole of the water present in the sample is united with the colloids in the gel form, such a conclusion being
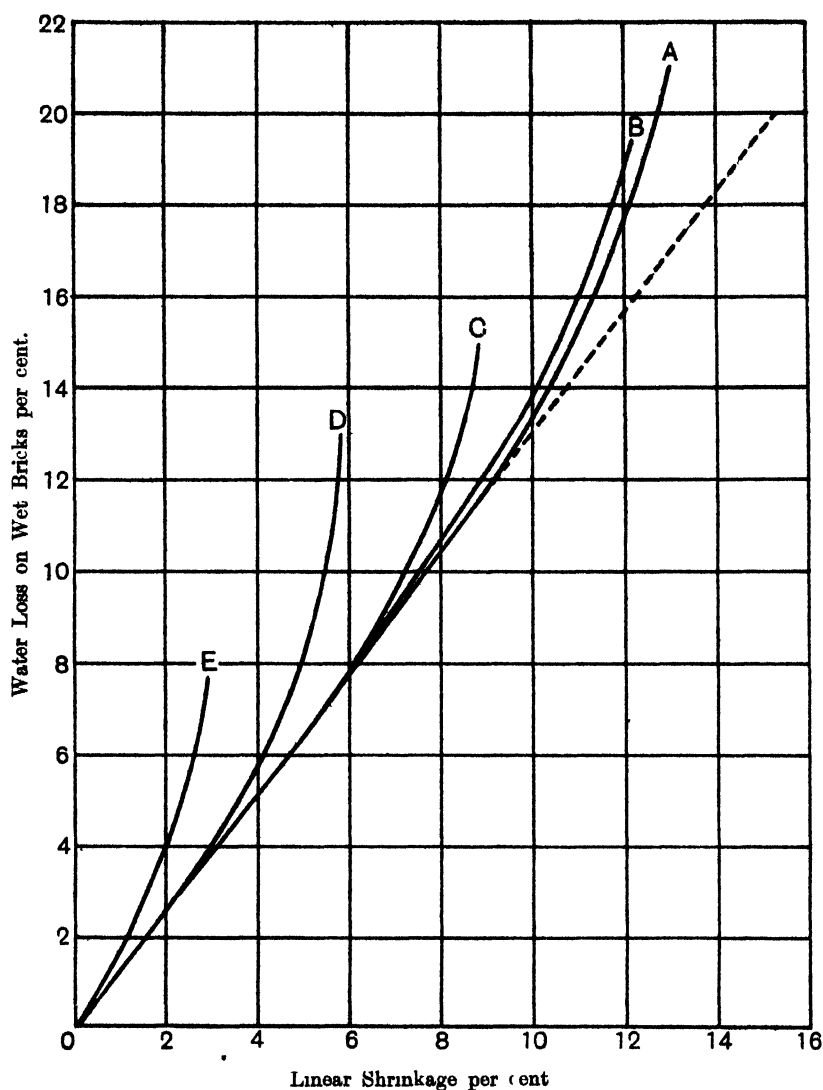
Fig 3.

indicated from the initial form of the curve and from the fact that varying amounts of water are required to be added to given weights of soil in order to produce maximum plasticity.

At the point of maximum plasticity the gel skeleton will ramify throughout the soil block surrounding in its meshes each one of the constituent soil particles. As the gel loses water by evaporation it will contract, and in contracting will tend to draw together the particles of the soil in its meshes.

In any event internal friction and the inertia of the soil particles will exert a certain degree of resistance to contraction, but in the earlier stages of drying, owing to the fact that the distances separating the particles are relatively large and the colloidal films relatively thick, the internal resistance will be very small and approximately constant.

As contraction proceeds however the constituent particles will approach nearer to one another and internal friction will in consequence tend to increase. Eventually a point will be reached when the effect of internal friction will become sufficiently pronounced to exert a perceptible retarding effect on the contracting force due to the gel, and this will be evidenced by a diminution in the rate of contraction in relation to the corresponding rate of water loss, and by a progressive departure from the straight line relationship. In the end the resistance due to these causes will become equal and opposite to the pull of the contracting gel, when further shrinkage will cease and the continuity of the gel skeleton within the block will become ruptured.

It is to be observed that in such an event, even after shrinkage has ceased, the gel condition continues to be maintained inside the block and will continue to lose water in the same way as previously, but as the continuity of the gel skeleton throughout the block has become broken no further shrinkage is evident.

That this is the case is clearly brought out when the total moisture contents of the soils are compared with the proportion of the total water present which has been evaporated when shrinkage ceases.

This is shown in the following figures which give the mean total moisture contents of each of the soils examined per cent. on the dry soil and the percentage of the total which has been evaporated at the time when shrinkage ceases.

	Mean moisture content per 100 grms. of dry soil	Per cent. of total moisture content evaporated when shrinkage ceases
A.	42·9	70·0
B.	40·5	67·0
C.	27·0	64·0
D.	25·0	61·0
E.	20·0	52·5

In any particular case the point at which the internal forces opposed to the pull of the contracting gel begin to make themselves felt must depend in large measure on the aggregate thickness of the colloidal film, that is to say on the amount of colloids present in the sample, and this will also govern the total contraction recorded.

Up to the present the relationship between contraction and water loss has been considered purely in respect of the linear contraction. It is now convenient to discuss the connection between linear and cubical contraction and the corresponding water loss.

The cubical contraction can be calculated from the observed linear contraction by means of the formula

$$C = \left(3a - \frac{3a^2}{10^2} + \frac{a^3}{10^4}\right),$$

where $C =$ the cubical contraction per cent.,

$a =$ the linear contraction per cent.

It is to be observed that with quantities of the order of magnitude in question the terms $-\dfrac{3a^2}{10^2} + \dfrac{a^3}{10^4}$ are not sufficiently small as to be negligible in comparison with the term $3a$, so that the relationship between linear and cubical contraction is not the simple linear function which with sufficient accuracy is usually assumed to exist in the case of linear and cubical expansion of solids under the influence of heat.

In point of fact so soon as the value of a reaches appreciable proportions the ratio $\dfrac{\text{Cubical contraction}}{\text{Linear contraction}}$ diminishes rapidly with increases in the value of a.

On the other hand in the earlier stages when the value of a is small the relationship does not depart very greatly from constancy.

If we examine the figures obtained in the case of Example A (in which the linear relationship between linear contraction and water loss has been shown to persist to the furthest point) and calculate the cubical contraction corresponding to the linear shrinkage up to a value of 9 per cent. for the linear shrinkage (at which point departure from the linear relationship becomes evident owing to causes already discussed), we obtain the following values:

Linear contraction per cent.	Cubical contraction per cent.	Water loss per cent. on wet brick
2·0	5·9	2·6
4·0	11·5	5·2
6·0	16·9	7·7
8·0	22·1	10·3
9·0	24·6	11·6

If the above figures are plotted it will be seen that the departure from the linear relationship is not marked.

When we go on to consider the relation between the actual weight of water lost and the corresponding cubical contraction, we are met with the difficulty that in the results adduced, some variation will be experienced owing to the fact that the density of the wet bricks will not be an absolutely constant quantity, owing to variations in the density of the constituent soil particles and in the content of water.

Actual measurements indicate that in the case of the soils examined the density of the wet blocks ranged between 1·95 and 2·1; if we assume as an approximation a mean density of 2·0 for the wet block and compare the actual weight of water lost per unit of cubical contraction for a block which when wet measures 100 c.c., in the case of Example A, over the range of values just examined, we arrive at the following data:

Water loss	Cubical contraction
5·2	5·9
10·4	11·5
15·5	16·9
20·6	22·1
24·6	23·2

Taking into account the fact that in calculating the cubical contraction any errors inherent in the linear measurement are multiplied nearly threefold, it appears reasonable to assume that, under the conditions of the experiment the cubical contraction is equal to the volume of the water lost by evaporation (when contraction is not influenced by internal friction among soil particles), and that in the case of a pure colloidal clay this linear relationship may be expected to hold good until shrinkage ceases altogether.

It follows from the foregoing that the relationship between linear contraction and water loss is not strictly linear in character and as the effect observed grows in magnitude the departure from the straight line relation will become more and more marked.

Conclusions.

1. In soils which have been moistened to the point of maximum plasticity the whole of the water probably exists in union with the colloidal material present in the soil in the form of a gel.

2. The gel occupies the whole of the interstitial spaces of the soil and as it loses water by evaporation draws together the soil particles in its meshes.

3. The normal relation between contraction and water loss in these circumstances appears to be that the cubical contraction observed to take place is equal to the volume of water evaporated from a given quantity of soil, provided that contraction proceeds unrestricted by internal friction among the soil particles.

4. In normal soils contracting in this way a point is reached at which internal friction among the soil particles offers sufficient resistance to the contracting pull due to the gel as to cause a progressively increasing departure from the normal relationship. The point at which this departure occurs and the magnitude of the total shrinkage observed appears to be a function of the amount of colloidal clay contained in any particular example.

5. The relation between cubical and linear contraction is in accord ance with the equation

$$C = \left(3a - \frac{3a^2}{10^2} + \frac{a^3}{10^4}\right)$$

PART II.

THE RELATIONSHIP BETWEEN CONTRACTION AND THE PERCENTAGE OF COLLOIDAL CLAY

Additional information concerning the process of shrinkage can be obtained from the study of the amounts of internal pore space existing in blocks of soil prepared in the manner already indicated, which have been allowed to contract to the full extent, and from which any residual water has been removed by subsequent drying to constant weight at 110° C.

EXPERIMENTAL.

The method adopted in performing this measurement consisted in covering the dried and weighed blocks with a coating of paraffin wax applied in a series of thin layers by means of a brush until the blocks had been rendered thoroughly waterproof. The blocks were then weighed again in air, the difference between the dry weight of the blocks and that of the waxed blocks giving the weight of the wax coating.

Subsequently the blocks were suspended from the hook of a balance and weighed in distilled water at a known temperature, and from the difference between the weight in air and the weight in water the volume of the waxed blocks calculated.

The specific gravity of the wax employed for coating the blocks was determined experimentally and found to be ·8995 and from·the previously ascertained data the volume of the wax coating was calculated and the nett volume of the dried blocks found by deduction.

Subsequently the apparent density of the blocks was calculated from the data obtained above, the actual density of the soil under examination determined experimentally by means of the specific gravity bottle, and the per cent. pore space in the block calculated by the formula

$$P = \frac{D_1 - D_2}{D_1} \times 100.$$

When P is the percentage of pore space in the block D_1 is the true density of the soil, and D_2 the apparent density of the soil block.

On these lines the internal pore space was determined on contracted and dried blocks of each of the soils A to E and also on three other soils designated F, G, and H.

The results of these are given below; the data are the means of a number of repetitions of each measurement which gave closely agreeing results.

Soil	Linear contraction	D_2	D_1	Mean pore space per cent. by volume
A.	13·0	2·03	2·32	12·5
B.	12·2	1·99	2·30	13·5
C.	8·8	1·97	2·37	16·9
D.	6·0	1·90	2·39	20·5
E.	2·9	1·84	2·41	23·6
F	6·9	1·85	2·30	19·6
G.	2·0	1·86	2·51	25·9
H.	1·0	1·82	2·52	27·7

The data show a regular increase in the pore space corresponding to the observed decrease in the linear and cubical shrinkage and when the values obtained for the pore space are plotted against the corresponding values for the shrinkage the experimental points are found to lie very approximately on a straight line (Fig. 4).

If this curve is extrapolated to cut the vertical ordinate corresponding to the shrinkage per cent. it is found that intersection takes place at a value for the shrinkage of 23·5 per cent.

The other extremity of the curve cuts the horizontal ordinate at a point corresponding to a pore space of approximately 28 per cent., which value approximates to that of the pore space which is found to exist in uncontracted coarse sand of uniform texture.

Especial interest centres in the point at which the curve cuts the vertical ordinate; this occurs when the value of the linear shrinkage is equal to 23·5 per cent. At this point the pore space will possess a value of zero. Now if it is assumed that the physical condition of pure dried colloidal clay approximates to that which occurs in continuous matter (such an assumption being presumably justifiable since the dimensions of colloidal particles though considerably submolecular
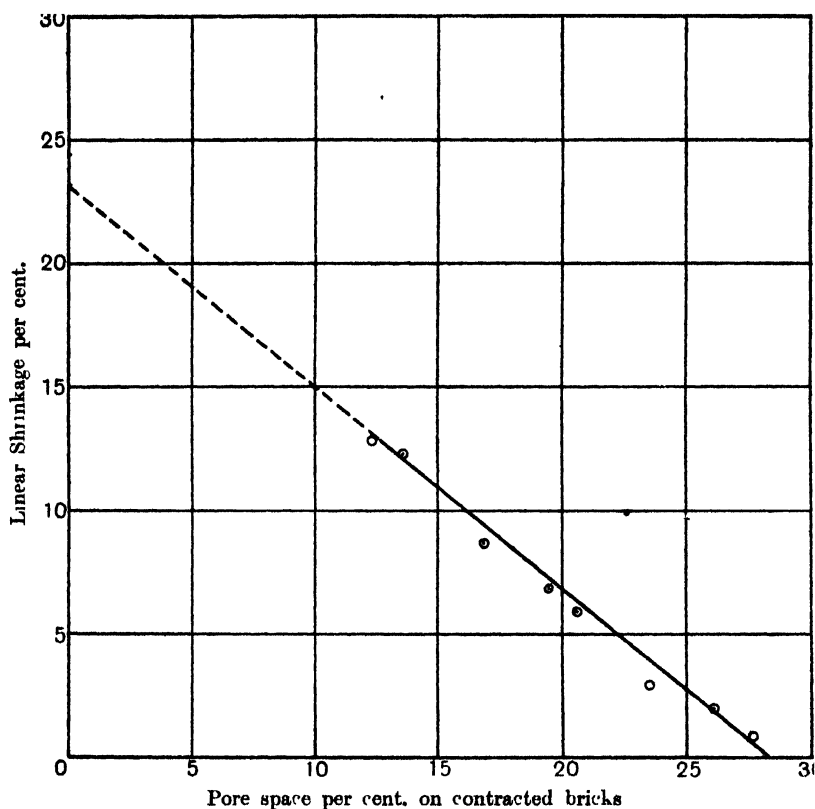
Fig. 4.

nevertheless commence to approach the molecular order of magnitude), we arrive at the figure obtained by extrapolation as a possible limiting value for the shrinkage of pure colloidal clay.

Such an assumption can only be regarded as approximate since the distance through which the curve has been extrapolated is considerable; on the other hand by utilising the value so obtained it is possible to

draw interesting deductions regarding the amount of colloidal clay contained in any sample of soil from a knowledge of the linear shrinkage.

It has already been shown that the observed linear contraction in the case of any particular soil depends directly on the content of colloidal clay, consequently it is obvious that if the maximum contraction exhibited in the case of pure colloidal clay is known it should become readily possible to calculate the proximate content of colloidal clay in any particular example. To effect such a calculation it will be merely necessary to multiply the observed linear shrinkage per cent. by the reciprocal of the limiting value for the shrinkage of colloidal clay and by 100.

The value of this factor on the basis of the above result is 4·25. Calculations performed in this manner will of course be only approximate but at the same time they are capable of comparison with known data obtained by other means.

It is a well-known defect of the ordinary method of physical analysis that while they are capable of effecting the separation of the coarser particles of the soil with considerable refinement of accuracy they do not give any exact indication of the amount of colloidal material contained in the soil, a factor which profoundly affects its agricultural value. In such physical separations the whole of the colloidal material is contained in the four fractions designated silt, fine silt, clay and organic matter; usually the amount of colloidal material contained in the silt fraction is small but in the writer's experience, especially in the case of soils containing much colloidal material, it is impossible completely to free the silt fraction from colloidal matter. In the fine silt and clay fractions the bulk of the colloidal material is always contained, but on the other hand it frequently happens that much material of a non-colloidal nature is also brought down with the colloidal material, and it is impossible by the ordinary methods of physical analysis to give expression to the actual amount of colloidal material present.

From the foregoing it is obvious that the calculation of the content of colloidal clay by means of a factor of the type indicated above, is capable of being checked inasmuch as, the whole of the colloidal material being contained in the fractions indicated, the total content of colloidal clay cannot exceed the total content of the four fractions in question and should be generally less. In point of actual practice since the bulk of the colloidal material is contained in the fine silt and clay fractions, it is sufficient for purposes of comparison to compare the content of colloidal clay calculated from the observed linear shrinkages and the

combined contents of fine silt and clay as determined by the methods of physical analysis.

In the following table these values have been calculated in the case of samples A to E referred to in the first part of this paper and also in the case of 16 additional samples of soil drawn from different parts of the Leeward Islands Colony. In this connection it is well to state that as the shrinkage determination has been performed on the fine earth samples of the soils in question, from which all particles having a diameter larger than 1 millimetre have been removed, the contents of fine silt and clay have in consequence been recalculated to the fine earth basis.

No.	Linear shrinkage per cent.	Content of Fine Silt and Clay as determined by physical analysis per cent.	Content of colloidal clay calculated from linear shrinkage per cent.
A	13·0	59·3	55
B.	12·1	60·6	51
C.	8·8	45·0	37
D.	6·0	42·1	25
E.	2·9	33·5	12
1	2·0	32·3	9
2	2·7	25·0	11
3	3·1	28·6	13
4	4·0	32·1	17
5	4·5	28·3	19
6	4·7	46·4	20
7	5·0	46·4	21
8	6·0	36·4	25
9	7·0	•35·6	˙30
10	9·0	44·5	38
11	10·0	63·1	43
12	11·4	69·5	48
13	12·0	61·7	51
14	13·0	65·5	55
15	14·0	63·0	60
16	15·0	70·5	64

Examination of the above results will show that in all cases the contents of colloidal clay, as calculated from the linear shrinkage by means of the employment of the factor in question, are less than the total content of fine silt and clay as determined by physical analysis, thus strengthening the conclusion already drawn; it is however interesting to observe that in the case of certain soils notably A, B, and Nos. 14, 15 and 16, the contents of colloidal clay approach very closely to the total content of fine silt and clay; it is to be remarked that in the case of all these soils they are particularly difficult to work especially in very wet or very dry weather.

A further approximate check on the validity of these deductions is capable of being effected if the fine silt and clay fractions of soils of known shrinkage are isolated, and separate determinations of the shrinkage of the separated fine silt and clay fractions carried out, since if the assumptions are correct and the approximate proportionality existing between the content of colloidal clay in the soil itself together with the content of fine silt and clay is known then, since the bulk of the colloidal material is separated in the fine silt and clay fractions, by the employment of the factor in question it becomes possible to calculate a theoretical value for the shrinkage which should be exhibited by the separated fine silt and clay fractions and to compare the figure so obtained with the result obtained by actual measurement.

This has been done in the case of Examples A and B and the results are given below:

	Actual shrinkage observed in the case of the fine silt and clay fractions	Calculated figure
A.	20·0 per cent.	21·8 per cent.
B.	18·2 „	19·8 „

The agreement between the calculated and the theoretical values in both these cases is sufficiently close to afford important confirmation of the conclusion already arrived at especially when it is borne in mind that the separation of the colloidal clay in the fine silt and clay fractions is not complete inasmuch as some will remain in the silt fraction.

The deductions arrived at in the foregoing pages indicate that by the employment of a factor of the value indicated it is possible to calculate the approximate content of colloidal clay in soils from a knowledge of the shrinkage. Such calculations are of course only approximate but at the same time they would appear to be capable of affording information of considerable value to the soil analyst apart from data obtained by the ordinary methods of physical analysis which as has been shown do not afford a complete insight into the character of the soil, inasmuch as they give no expression for the amount of colloidal material therein existing.

It is hoped that in a subsequent paper it may be possible to trace the relationship exhibited between the values for the content of colloidal clay calculated from the shrinkage and data derived from a study of the adsorption coefficient of different soil types.

SUMMARY.

1.   By determination of the internal pore space in blocks of soils and comparison with the observed value for the linear shrinkage it is found that a linear relationship appears to exist between the two values.

2.   By extrapolating the curve thus obtained an approximation for the limiting value of the shrinkage in the case of pure colloidal clay is arrived at amounting to approximately 23 per cent.

3.   On this assumption it becomes possible to calculate the approximate content of colloidal material in any soil from a knowledge of the linear shrinkage.

4.   Results are adduced, showing the values obtained for the shrinkage in the case of separated fine silt and clay fractions in the case of two soils of known shrinkage and physical composition, and compared with the values calculated from previous assumptions.

5.   The results of the calculation of the content of colloidal clay in the foregoing manner in the case of 16 Leeward Islands soils are appended.

DISSOLVED OXYGEN IN RAIN-WATER.

By ERIC HANNAFORD RICHARDS.

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FROM the agricultural point of view the amount of oxygen carried into the soil as gas dissolved in rain has considerable interest. Since all the oxygen required either for the bacterial activities of the soil or for the root aeration of growing crops must first pass into the dissolved state, the supply of this essential element in a form immediately available must exercise a proportionately beneficial effect on the manufacture of plant food or on the growing crop. Recent work on the decomposition of organic matter in the soil[1] has shown that rainfall is one of the chief factors controlling bacterial activity, but that its influence is due to something more than the supply of water. The oxygen dissolved in the rain appears to be the missing factor. As very little systematic work seems to have been done in this direction, estimations of dissolved oxygen in rain at Rothamsted were made from time to time during the year 1915.

It is natural to suppose that rain should be saturated with dissolved oxygen, the actual amount varying with the temperature at which the rain was condensed. As it is difficult to determine this temperature the comparatively few observers who have studied the gaseous content of rain, have usually been content to record the volume of gas found and the temperature of the rain-water when collected.

As long ago as 1800 Senebier[2] thought that sufficient attention had not been paid to the quantity of oxygen gas contained in rain and quotes Hassenfratz[3] who had found more dissolved oxygen in rain-water than in water from the Seine, concluding from this that rain-water was more beneficial to plants than other waters.

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[1] E. J. Russell and A. Appleyard, this *Journal*, **7**, 1915, 24; also this vol. p. 385.

[2] *Physiologie Végétale*, vol. III, p. 88, Geneva, 1800.

[3] *Hermbstadt's Archiv der Agriculturchemie*, vol. I (Berlin, 1804), p. 396.

Humboldt and Gay-Lussac[1] found that the gas from aerated distilled water contained 32·9 per cent. of oxygen compared with 31·0 per cent. in rain-water. Gérardin[2] in two papers records figures which are mostly below saturation. Reichard[3] gives a series of analyses made at Jena of which only one sample taken in January, at 4° C., is saturated with oxygen. At the other extreme a sample in July, at 24° C., is only 62 per cent. saturated.

The rain was collected at Rothamsted in two large glass funnels 10 inches diameter, the tubes reaching to the bottoms of two narrow-mouthed bottles of about one litre capacity. They were supported in a stand so that the rims of the funnels were 2 feet 6 inches above ground level. At first the two collectors were used in duplicate to check the results obtained, but as the figures for the two samples were invariably identical, a layer of liquid paraffin was subsequently placed in one bottle sufficient to cover the end of the funnel tube and protect the surface of the rain as it collected from contact with the air.

No determinations of dissolved oxygen were made on rainfalls under 0·30 inch, as the size of the funnels was too small to yield the necessary amount of sample. The bottles were of course emptied after any rain and both these and the funnels were frequently cleaned.

TABLE I. *Dissolved Oxygen in mixtures of 1 per cent. Belfast Sewage with Tap-water and Sea-water kept in full stoppered bottles.*

Dissolved oxygen in c.c. per litre at N.T.P. Temperatures in ° F.

	When mixed at			After 5 days at			After 14 days at			After 42 days at		
	60°	70°	80°	60°	70°	80	60°	70°	80°	60°	70°	80
Tap-water mixture ·												
Oxygen (gasometric)	6·2	6·0	5·4	5·0	4·1	3·0	4·2	4·2	0·9	2·9	7·1	0·2
Oxygen (Winkler)...	6·6	5·9	5·5	5·0	4·0	3·0	4·0	4·2	0·9	2·9	7·4	0·1
Sea-water mixture :												
Oxygen (gasometric)	5·5	5·1	4·7	3·9	3·5	2·7	3·3	3·2	1·6	2·7	2·5	0·2
Oxygen (Winkler)...	5·7	5·4	5·0	4·0	3·6	2·8	3·2	3·1	1·5	2·5	2·3	0·1

Winkler's[4] method was used for estimating the dissolved oxygen. The author has had many years' experience of this method and has always found it entirely satisfactory. When properly carried out it gives results in close agreement with the best gasometric determinations. Table I gives some comparative estimations made by Prof. Letts

[1] "Mémoire sur l'Eudiometrie," *Journal de Physique,* 1805
[2] *Compt. Rend.* **75,** 1872, p. 1713, and **81,** 1875, p. 989.
[3] *Archiv Pharm.* 1875, **206**; III, vol. VI, p. 193.
[4] *Berichte,* vol. XXI, pp. 2, 843 (1888).

and the author in the course of an investigation for the Royal Commission on Sewage Disposal[1].

The results of a year's observations at Rothamsted are set out in Table II. It will be noticed that, taking Dittmar's[2] figures for distilled water saturated with air as a standard, the samples of rain-water collected at temperatures below 15° C. are practically all only slightly under saturation (average 93 per cent.), but the samples taken in the summer months are all less aerated (average 85 per cent.). The summer samples collected by Reichard are also all considerably below saturation.

The dissolved oxygen content of the samples collected under paraffin was usually very slightly below that of the samples exposed to air. This difference may be due either to the taking up of oxygen from the air by the exposed sample while it accumulated in the collecting bottle, supposing it to be below saturation when it fell, or to absorption of dissolved oxygen by biological oxidation of organic matter brought down by the rain in the sample collected under paraffin. Possibly both actions were sometimes responsible for the difference observed. There is no doubt that the second process does occur quite rapidly in the case of samples collected from the first rain after a period of drought. On August 14, 1916, after three weeks without rain the sample exposed to air gave 0·62 parts per 100,000 and the paraffin insulated sample 0·61 at 17·5° C. or only 63 per cent. saturation. This rain was visibly dirty and there is no doubt that some absorption took place during the twenty hours that elapsed between the fall of the first drops of rain and the analysis of the sample. On the other hand samples collected during heavy rain on August 3, 1915, and analysed at once showed only 88 per cent. saturation. There was no time for any absorption of oxygen here even if the sample had been dirty which was not the case as there had been frequent rains in the two weeks immediately preceding.

To see how much dissolved oxygen rain-water would take up in the maximum time elapsing between collection and analysis, a sample from a heavy rainfall in the early morning was siphoned into six bottles at 11 a.m. the same day. This experiment was designed to show (1) if there was any absorption of oxygen in 24 hours at 14° and 25° C., (2) if the paraffin was responsible for any of the deficiency found in the samples collected under a layer of oil, and (3) if rain falling at night at a relatively low air temperature and kept in the collecting bottles

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[1] *Royal Commission on Sewage Disposal, 7th Report*, vol. II, Appendix, p. 111 (1911).

[2] *Challenger Reports, Physics and Chemistry*, vol. I, p. 160.

TABLE II. *Dissolved Oxygen in Rain-water samples collected at Rothamsted in 1915.*

Oxygen expressed as parts per 100,000 by weight.

Date, 1915	Temperature in °C. at time of collection	Dittmar's figure for distilled water saturated with air	Oxygen in samples exposed to air	Percentage saturation	Oxygen in samples collected under paraffin	Percentage saturation	Notes
Jan. 22	4·5	1·30	1·02	78	—	—	Rain began 10 a.m. Collected 5 p.m.
,, 23	0·8	1·43	1·24	87	—	—	Very heavy rain at night.
Feb. 2	10·0	1·14	1·11	97	—	—	Rain in afternoon and evening.
,, 3	10·7	1·12	1·15	103	—	—	Drizzling rain.
,, 6	7·5	1·21	1·15	95	—	—	Heavy rain at night.
,, 8	4·0	1·32	1·12	85	—	—	Heavy rain, probably warmer than 4°.
,, 9	5·5	1·27	1·20	94	—	—	Heavy rain at night.
,, 17	7·5	1·21	1·17	97	—	—	Heavy rain, falling when collected 10 a.m.
,, 24	5·0	1·28	1·29	101	—	—	Melted snow only. Naturally melted in funnels.
March 24	12·0	1·09	1·05	96	—	—	Rather fine rain.
April 7	9·0	1·17	1·11	95	—	—	Squalls at long intervals.
,, 13	7·5	1·21	1·17	97	—	—	Drizzle on 12th followed by heavy rain at night.
July 7	16·5	1·00	0·79	79	0·73	73	Thunderstorm 11 p.m. on 6th.
,, 23	15·0	1·03	0·85	83	0·77	75	Thunder showers.
,, 26	19·0	0·95	0·83	87	0·79	83	Heavy showers.
Aug. 3	16·5	1·00	0·86	86	0·77	77	Squalls on 2nd.
,, 3	14·0	1·05	0·92	88	0·92	88	Thunderstorm 2–4 p.m. Collected 6 p.m.
Sept. 29	8·5	1·19	1·05	88	—	—	Heavy cold rain for 12 hours.
Oct. 25	8·0	1·20	1·07	89	0·97	81	Cold rain followed by drizzle.
,, 28	9·0	1·17	1·11	97	1·13	97	Raining when collected 11 a.m.
Nov. 1	9·0	1·17	1·08	92	1·08	92	Steady rain for 24 hours.
,, 10	7·0	1·22	1·10	90	1·07	88	Squalls on 9th.
,, 12	8·0	1·20	1·17	97	1·16	97	Heavy rain all night.
,, 13	4·0	1·32	1·17	87	1·15	87	Gale with cold rain.
Dec. 1	10·5	1·13	1·10	97	1·08	96	Squalls same morning. Collected 3 p.m
,, 6	9·5	1·16	1·03	89	1·03	89	Heavy rain.

until its temperature had risen considerably, as was the case in the summer mornings, would lose oxygen owing to decrease in solubility.

On the last point an experiment[1] made by the author in Dr McGowan's laboratory showed that good tap-water saturated with air at 7° C. could be incubated for six days at 18° without appreciable loss of oxygen. It was of course considerably supersaturated at the higher temperature, but as long as the bottle is not shaken and no fermentation occurs there is no appreciable loss of oxygen.

In the experiment with rain one bottle to be kept at each temperature was tightly stoppered and the other had a layer of the same paraffin, as used in the collection of samples, covering the water just below the neck. This experiment gave the following figures:

	At start	After 24 hours			
		at 14°		at 25°	
	10°	Stoppered	Paraffined	Stoppered	Paraffined
Dissolved oxygen in parts per 100,000	1·11 1·12	1·11	1·12	1·06	1·06
Saturation (Dittmar)	1·14	1·05		0·85	

Dissolved oxygen.
Parts per 100,000
by weight.

- - - - - - Dissolved oxygen in distilled water saturated with air at temperature of rain-water.

———— Dissolved oxygen in rain-water.
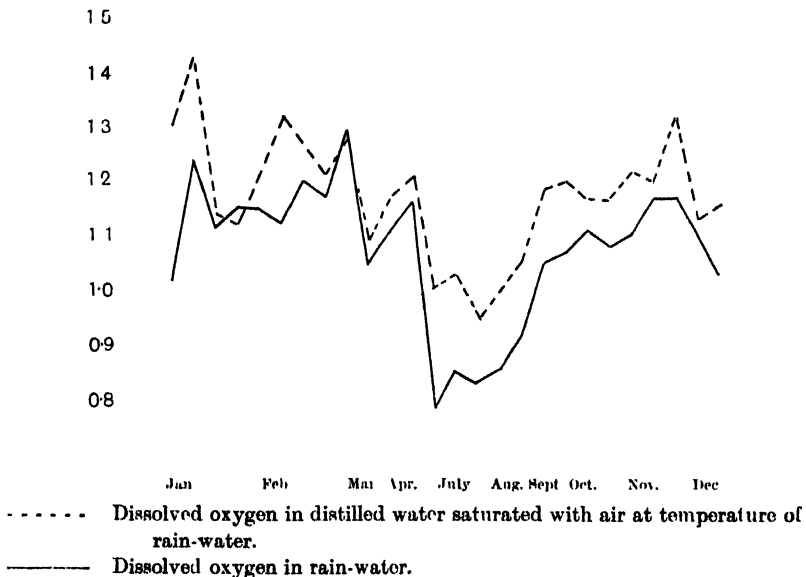
Fig. 1.

[1] *Royal Commission on Sewage Disposal, 8th Report*, vol. II, Appendix, p. 96 (1913).

These results show (1) that with normal clean rain there is no absorption of oxygen in 24 hours; the small difference at 25° is almost certainly due to the 42 per cent. supersaturation; (2) that the paraffin has no more action than the glass stopper; (3) that rain-water, like tap-water, if it is not shaken, can become strongly supersaturated without loss of oxygen.

Fig. 1 shows the dissolved oxygen in the rain-water samples arranged in the order of collection during the year, compared with Dittmar's figures for distilled water saturated with air at the temperatures of the rain-water when collected.

Dissolved oxygen
Parts per 100,000
by weight

- - - - - Dissolved oxygen in distilled water saturated with air at temperature of
         rain-water when collected.
———— Dissolved oxygen in rain-water.
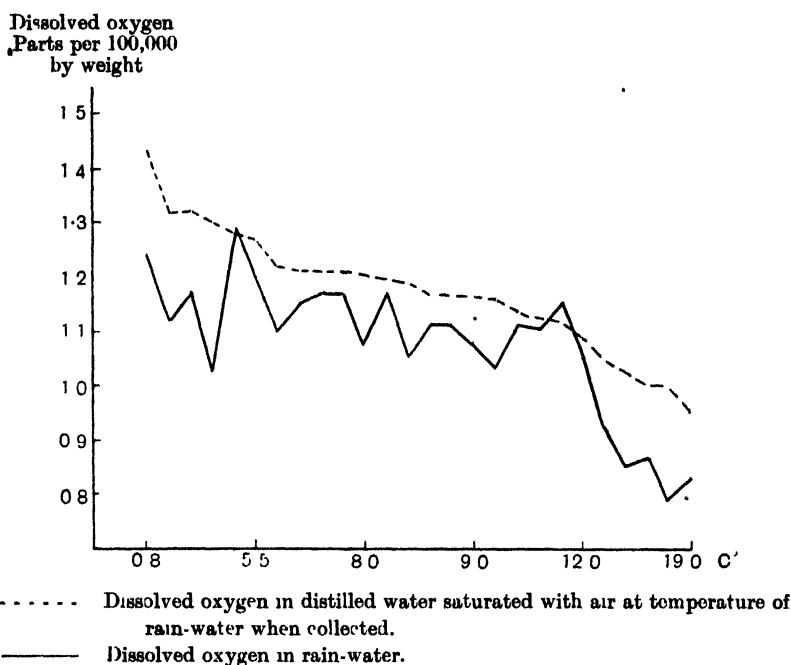
Fig. 2.

In Fig. 2 the samples are arranged in order of temperature when collected. It will be noticed that the first four samples, collected at temperatures below 5° C., are considerably under saturation. In these cases the rain as it fell was at a temperature perhaps 5° above that which it assumed after standing some hours at a much lower air temperature. The interval between the rain falling and the collection of the sample was long enough to lower the temperature of the rain-water in the bottle but not long enough for the exposed sample to saturate itself

with air at the lower temperature. With a small undisturbed surface relative to volume of water aeration is a very slow process. The observations made by Passerini[1] on the difference of temperature of air and rain at the moment of falling, show that in Italy rain is normally about 2° cooler than the air, but occasionally the difference is as much as 10° C.

It seems to be clear that summer rain, unlike that which falls during the rest of the year, is not fully saturated with oxygen. It is difficult to understand the reason for this. The relative temperatures of the rain clouds and of the air at the ground level in summer and winter, if they have any influence at all, should produce a result exactly opposite to that actually found, i.e. summer rain ought to be supersaturated when collected.

We may conclude that rain-water is very nearly saturated with oxygen when its temperature as collected is below 15° C. as is the case for about nine months in the year in this climate. When the temperature of the rain is above 15° C., the dissolved oxygen is always below saturation, occasionally as much as 25 per cent.

The author wishes to acknowledge the help given him with the literature of the subject by the late Dr N. H. J. Miller.

[1] *Bolletino della Scuola Agraria di Scandicci*, vol. I, 1893, p. 95; also vol. II, p. 65.

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STUDIES ON THE PALÆOZOIC SOILS OF NORTH WALES.

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Introductory.

THE soils of North Wales have been studied by the writer during the past four and a half years in field and laboratory. A number of types have been recognised and studied. Further work is necessary to elucidate the properties of the types already identified and to establish fresh types. The purpose of the present paper is to give an account of the soils most typical of the area, namely the soils derived from material of the Pre-Cambrian, Cambrian, Ordovician and Silurian formations in the counties of Anglesey, Carnarvon and Denbigh[1]. In this group are included soils derived from the weathering of local glacial drift, and the associated sandy, alluvial and peaty soils. The soils formed of newer materials, including later Palæozoic soils and Glacial drift of external origin may form the subject of a separate paper.

A number of soils of the Geological period in question have already been described by the writer elsewhere[2]. Otherwise, little or no work has been done on the Palæozoic soils. The bulk of the experimental work on soils in England has been on soils of Jurassic and later ages.

Physical Features and Climate of North Wales.

North Wales is a country of mountains, hills and valleys. The character of its surface and the proximity of the sea give character to its climate, soil and agriculture. The accompanying orographical map will give an idea of the structure of the area. It will be seen that the

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[1] A complete survey of Flintshire soils has been made by Mr C. F. Hill, B.Sc., formerly research student of this college. The Flintshire soils are mainly of later age than those discussed in the present paper.

[2] *A Survey of the soils and Agriculture of Shropshire*, Shrewsbury, 1912.

island of Anglesey, coastal Carnarvonshire, the vales of Conway and Clwyd, and the English border, lie generally below 600 feet. In Carnarvonshire there is a fairly sharp transition from the coastal lowlands to the wild mountainous tracts of Eryri or Snowdonia. In Denbighshire and Flintshire the transition is more gradual and there is an almost complete absence of rugged mountain land such as is found in Carnarvonshire.

NORTH WALES. Orographical.

Altitudes in feet		Altitudes in feet	
☐	0—600′	■	1000—2000′
▨	600—1000′	▨	Above 2000′
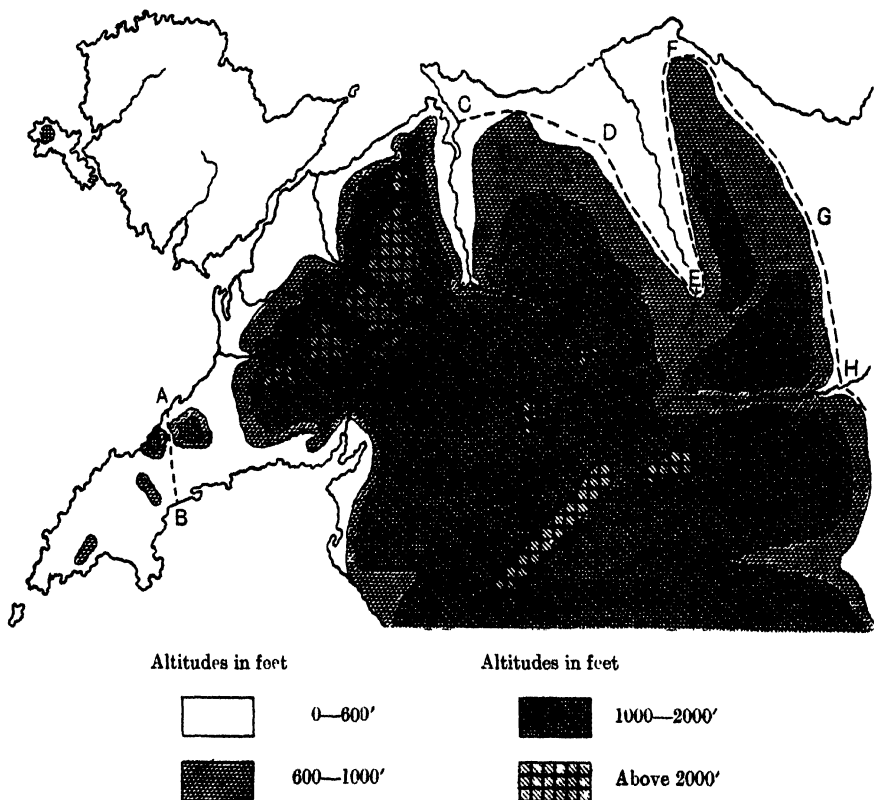
The lowland districts have, in general, extremely undulating outlines, showing in miniature the form of the more elevated areas. Flat stretches exist in the vales of Conway and Clwyd, the northern coastal fringe and along the Cheshire border.

The climate of North Wales is governed by the contour of the land and the proximity of the sea. The distribution of rainfall is shown on

the accompanying map which has been constructed from data in *British Rainfall*, 1887–1912. The connection between elevation and rainfall is strikingly seen on comparison of the orographical and rainfall maps.

NORTH WALES. Rainfall.

Mean annual rainfall

25 to 30 inches   35 to 40 inches   45 to 50 inches

30 to 35 inches   40 to 45 inches   50 to 100 inches

Over 100 inches
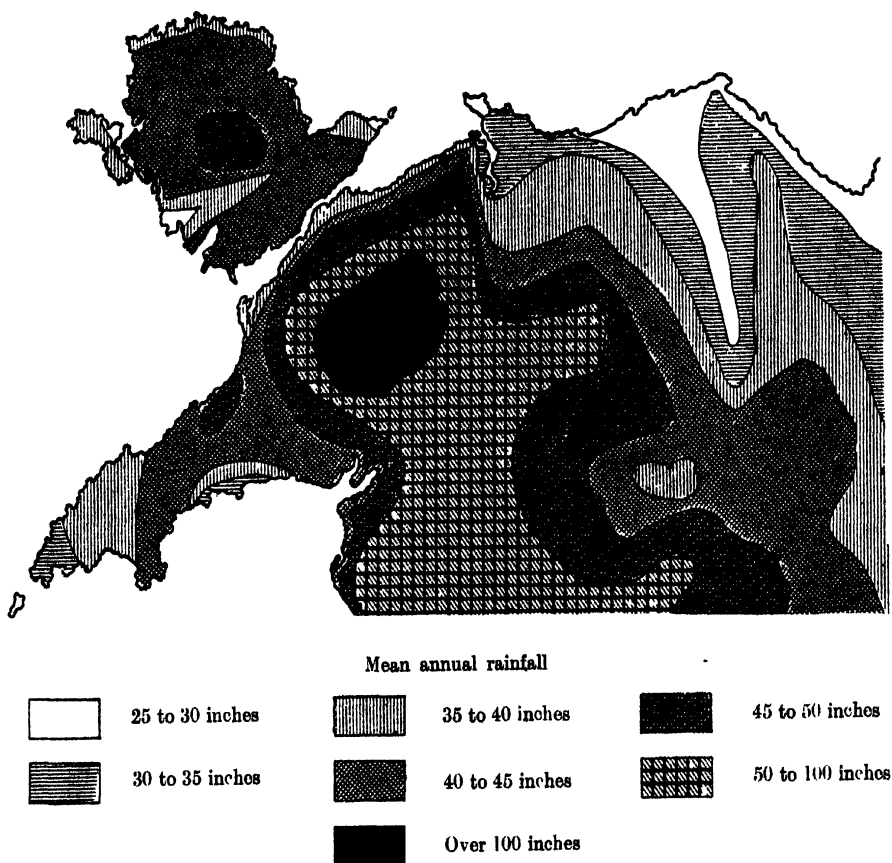
The writer estimates that in the four northern counties of Wales, of the total surface

7 %	has a mean annual rainfall of			25–30	inches
20 %	„	„	„	30–35	„
30 %	„	„	„	35–40	„
26 %	„	„	„	40–50	„
12 %	„	„	„	50–100	„
5 %	„	„	„	> 100	„

These figures are only approximate, but will indicate the general wetness of the North Welsh climate. Comparatively little cultivated land is found under annual rainfall greater than 50 inches.

Temperature records are somewhat scanty, but the general facts are fairly clear. The mean winter temperature varies from 39° F. to 42° F. and increases from East to West so that Anglesey and West Carnarvonshire are as warm in January as Devonshire. The elevated districts of the interior are naturally somewhat colder than the coastal districts. The average summer temperature is somewhat less than the average summer temperature of central and southern England.

High winds are prevalent along the sea coasts during the months from October to April.

To sum up, North Wales may be described as an area with high rainfall, cool summers and generally mild winters.

Geology.

Two main facts must be noticed in order to understand the geological structure of North Wales. (1) The rocks are mainly Palæozoic except in the Eastern portion where Triassic deposits occur under the drift, and (2) the whole of the area has been glaciated.

The Palæozoic rocks which underlie most of the area and whose resultant soils form the subject of this paper fall into three main groups.

A. The Pre-Cambrian metamorphic rocks of Anglesey. These rocks which cover three-quarters of the area of Anglesey consist of a series of schists and gneisses of variable composition. Rocks believed to be of the same age occur in the extreme west of Carnarvonshire but are generally obscured by drift. The Pre-Cambrian rocks rarely rise to more than 400 feet and in no case more than 750 feet.

B. The main part of Carnarvonshire consists of Ordovician strata. With these are associated enormous masses of igneous and volcanic rocks which form the core of the mountain area.

C. Ordovician and Silurian shales and flagstones occur without associated igneous rocks in Denbighshire and Flintshire, and form the greater part of the area of the former county. They are generally free from external drift.

Cambrian shales and slates occur in a broad strip parallel to the Menai Straits and in a few other places but do not give rise to any extensive area of soil. The other Palæozoic rocks of North Wales, including Old Red Sandstone and Carboniferous rocks, are of secondary importance from the point of view of the soil investigator.

Two glaciations have left their marks on the Geology of North Wales, namely the northern glaciation which flowed across the Irish Sea in a direction roughly N.N.E. to S.S.W. and the glaciation radiating from the Arenig and Snowdon mountains. The Northern glacier affected the whole of Anglesey and West Carnarvonshire and the other two counties up to approximately the 600 feet contour line. The drift deposits in Anglesey, although the result of northern glaciation, consist in the main of local material. In the other parts of North Wales affected by the northern glaciation the drift deposits are in the main of external origin. The drift deposits of the local glaciation, which are found in greatest profusion in Carnarvonshire, consist of local materials scraped down from the adjacent mountains[1].

The Welsh drift in Denbighshire and Flintshire is found in the bottoms of upland valleys and is nowhere so extensive as the local drift of Carnarvonshire.

Referring now to the orographical map, the lines *AB* and *CDEFGH* show the boundaries of the Palæozoic soils of North Wales. West of *AB* and north of *CDEFGH* the soils are mainly drift soils formed from material of later origin. It should be added that the Palæozoic area includes practically the whole of Anglesey and Denbighshire.

The soils discussed in the present paper are from this Palæozoic area which will be seen, by reference to the rainfall map, to be also the area of highest rainfall.

Palæozoic Soils.

In presenting an account of the types of soil encountered, the question of classification is naturally important[2]. The system of classification adopted will naturally depend on the material to be classified and the local conditions. Thus, the Russian soil investigators, Sibirtzev, Docuchaiev and others, use a classification based on climatic conditions because climate is the great controlling factor in the regions studied by them. In other regions, where the climate is relatively constant, the petro-geological classification first proposed by Fallou and adopted by Hall and Russell in their work in Kent, Surrey and Sussex, is most suitable. In an area of varying climate, geology

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[1] For an account of the Glacial Geology of Western Carnarvonshire, see T. J. Jehu, *Trans. Roy. Soc. Edin.* vol. XLVII, pt. 1, 2. The general outlines of the Geology of North Wales are given in Ramsay's Geological Survey memoir.

[2] See N. M. Tulaïkoff, this *Journal*, vol. III, pt. 1, p. 80; E. Ramann, *Bodenkunde*, p. 521; and Hall and Russell, this *Journal*, vol. IV, pt. 2, p. 182.

and terrain, neither of the last two systems is alone suitable. This is the case in the United States where a physical system of classification is used. Lastly, there is the system of classification on ecological and agricultural lines recommended by Hall and Russell[1]. This last system has been adopted as far as possible by the writer in North Wales. Its use is limited on the agricultural side by the operation of factors other than those connected with soil and climate. On the ecological side certain broad classifications can be made. Sand dune, moor, salt marsh and other formations can be distinguished in waste lands, but it is a matter of considerable difficulty to refer all cultivated lands to their original plant formations.

In classifying the soils of North Wales, genetic factors as well as the intrinsic properties of soils have been taken into consideration in addition to agricultural and ecological characters. By this means a provisional classification has been arrived at which may be modified by sub-division as more information is obtained. The system adopted will be best explained by the accounts of the various soil types.

The analytical methods adopted are those generally used in soil surveys in this country[2].

Table I shows the average percentage composition of each soil type. In order to give some idea of the dispersion of the results, the range is given corresponding to each average. Thus in the case of the Anglesey medium loam we see that the percentage of fine sand in the soil is 21·2 and the range 16·1 to 29·2. This means that in all the samples examined the percentage of fine sand was never less than 16·1, nor greater than 29·2. The range is of course a rather imperfect measure of dispersion but is the only·possible measure where the number of results is small. Where a larger number of results is available, more satisfactory measures can be used. The most direct for the purpose of showing the dispersion of soil analyses appears to the writer to be the interquartile range[3]. The method of obtaining it may be illustrated from an actual example. In the case of the Anglesey medium loam the percentages for fine sand in the soil are arranged in ascending order of magnitude. There are 26 observations. The lower quartile is given by the seventh value from the bottom of the list and the upper quartile

TABLE I. *Average percentage composition of soil types.*

| | Anglesey Medium Loam | | | | | | | Palæozoic Silt Loam | | |
| | Main type | | | Sandy sub-type | | Granite sub-type | | | | |
Soils	Average	Range	Interquartile range	Average	Range	Average	Range	Average	Range	Interquartile range
Fine gravel	8·3	1·9 –14·2	5·9 –10·7	5·1	4·4 – 7·7	8·7	6·4 –11·0	10·6	3·5 –26·1	7·3 –13·5
Coarse sand	18·0	7·7 –29·2	14·8 –21·8	50·0	44·0 –66·6	16·2	15·8 –16·8	8·0	2·2 –12·7	5·2 –10·6
Fine sand	21·2	16·1 –29·2	18·4 –22·6	15·8	10·9 –20·0	21·2	20·5 –21·8	14·1	8·2 –30·9	10·6 –16·5
Silt	13·6	9·4 –17·0	12·0 –15·2	9·6	5·5 –12·0	13·3	13·1 –13·4	14·1	6·9 –21·0	12·5 –17·2
Fine silt	18·7	13·2 –24·2	16·2 –20·5	9·1	5·8 –12·5	19·6	17·4 –21·7	28·8	20·7 –40·8	26·7 –32·7
Clay	5·0	1·9 – 9·4	4·0 – 5·9	2·4	1·2 – 2·9	7·7	5·3 –10·1	6·6	3·2 –11·6	5·7 – 7·9
Moisture	3·8	1·5 – 6·2	2·8 – 4·9	1·8	1·0 – 3·0	4·4	4·4 – 4·4	2·7	·9 – 4·0	2·2 – 3·9
Organic matter	8·7	5·0 –11·6	8·0 – 9·4	5·4	4·2 – 6·3	10·1	9·8 –10·5	12·8	7·2 –23·3	10·6 –13·8
Nitrogen	·29	·16– ·36	·27– ·31	·20	·16– ·21	·31	·31– ·31	·42	·25– ·73	·35– ·45
K_2O soluble in	·51	·34– ·61	·47– ·54	·49	·37– ·63	·79	·70– ·88	·80	·53– 1·20	·68– ·85
P_2O_5 strong HCl	·15	·05– ·28	·11– ·18	·12	·10– ·18	·19	·17– ·21	·185	·06– ·324	·14– ·21
Subsoils										
Fine gravel	11·3	4·3 –18·6	7·0 –15·3	6·1	2·5 – 9·6	12·8	12·3 –13·4	18·1	2·8 –35·5	7·8 –18·9
Coarse sand	17·7	8·7 –28·4	13·5 –21·7	48·9	31·0 –72·0	18·1	12·8 –23·4	8·6	2·0 –15·7	6·1 –11·0
Fine sand	22·2	16·6 –32·2	19·9 –24·3	18·3	11·0 –22·5	18·6	18·1 –19·1	13·6	4·9 –30·6	10·0 –14·9
Silt	14·7	10·1 –20·7	13·1 –16·5	10·4	6·2 –14·3	13·0	10·7 –15·4	17·4	10·6 –35·4	14·8 –20·1
Fine silt	17·4	11·2 –21·4	16·1 –19·4	9·8	3·2 –14·0	14·0	11·0 –17·0	25·4	18·9 –38·7	22·2 –29·1
Clay	6·5	2·5 –13·7	4·3 – 7·9	3·4	1·4 – 5·5	6·5	4·6 – 8·5	7·9	2·4 –17·1	5·0 – 9·2
Moisture	2·7	·8 – 6·0	2·2 – 3·4	1·6	·8 – 2·6	2·2	2·1 – 2·2	2·0	1·1 – 4·7	1·8 – 3·0
Organic matter	5·7	4·0 – 7·5	5·1 – 6·3	3·7	2·7 – 4·7	7·2	6·9 – 7·4	8·3	4·9 –15·6	·6·1 – 8·8

Carnarvonshire Stony Loam

Soils	Sub-type A		Sub-type B		Sub-type C		Sub-type D	
	Average	Range	Average	Range	Average	Range	Average	Range
Fine gravel	8·0	1·0 –10·7	14·1	11·8 –18·0	14·7	9·0 –18·9	12·0	11·4 –12·4
Coarse sand	9·5	4·1 –17·6	12·8	10·6 –14·6	21·2	19·8 –22·1	14·2	10·4 –17·2
Fine sand	21·6	17·6 –24·2	21·7	20·0 –23·2	20·4	19·1 –22·1	15·3	13·4 –16·1
Silt	14·8	10·9 –20·4	12·7	11·7 –14·0	9·9	8·7 –10·4	8·1	6·0 – 9·6
Fine silt	24·8	23·1 –26·5	18·2	16·7 –19·8	13·95	12·7 –14·7	21·3	20·6 –21·9
Clay	5·7	2·7 – 7·2	3·1	1·6 – 5·1	3·1	2·8 – 3·4	5·6	5·1 – 6·1
Moisture	3·2	1·0 – 5·1	3·4	1·0 – 6·2	3·1	2·3 – 3·9	5·0	3·6 – 7·2
Organic matter	13·0	7·8 –17·5	13·7	12·8 –15·3	13·9	11·9 –15·5	15·7	11·3 –20·3
Nitrogen	·48	·25– ·59	·47	·44– ·53	·55	·42– ·64	·51	·45– ·60
K_2O soluble in strong HCl	·59	·46– ·68	·59	·55– ·63	·80	·56– ·93	·35	·25– ·45
P_2O_5 soluble in strong HCl	·15	·07– ·22	·21	·18– ·23	·26	·19– ·31	·15	·12– ·17
Subsoils								
Fine gravel	6·1	1·0 –11·5	8·5	4·6 –14·7	32·0	19·8 –46·8	23·3	20·3 –24·8
Coarse sand	8·3	4·9 –13·0	11·7	9·7 –13·7	14·6	13·6 –17·4	16·5	13·8 –19·0
Fine sand	16·7	12·6 –25·5	25·4	19·1 –34·0	13·2	9·0 –18·4	8·2	6·0 – 9·7
Silt	24·1	14·3 –32·4	16·4	11·5 –19·5	7·4	1·4 –11·5	20·4	18·9 –23·8
Fine silt	23·0	11·3 –27·8	19·5	12·7 –23·4	12·7	9·5 –14·4	11·4	8·7 –13·3
Clay	7·8	5·4 –11·0	4·2	2·4 – 7·2	2·3	1·5 – 2·8	5·5	4·5 – 6·7
Moisture	3·1	·9 – 5·2	2·7	1·6 – 4·2	2·7	1·3 – 3·3	2·4	1·7 – 3·4
Organic matter	8·9	6·2 –11·6	9·9	9·3 –10·8	12·8	9·5 –15·6	7·3	6·3 – 8·2

TABLE I (*continued*). *Average percentage composition of soil types.*

Soils	Wind-Blown Sands		Alluvium					Peat			
			Estuarine		Fluviatile			Shallow peat		Deep peat	
	Average	Range	C 23	C 24	D 7	D 51	D 10	Average	Range	Average	Range
Fine gravel	1·2	·0 – 5·75	·0	·5	1·1	1·6	·7	—	—	—	—
Coarse sand	80·5	63·3 –94·3	15·6	18·0	12·4	3·0	·5	—	—	—	—
Fine sand	10·1	2·5 –20·0	21·2	69·3	20·7	11·6	2·5	—	—	—	—
Silt	1·95	·3 – 6·7	19·1	2·4	22·0	20·8	9·3	—	—	—	—
Fine silt	1·8	·3 – 6·6	23·4	1·4	25·9	45·6	57·6	—	—	—	—
Clay	·25	·0 – 1·2	10·3	·5	3·4	4·6	13·9	—	—	—	—
Moisture	·7	·4 – 1·5	2·1	·7	3·6	2·1	2·7	7·6	3·0 –12·2	7·6	7·0 – 8·3
Organic matter	3·1	1·7 – 5·5	6·9	4·0	7·6	9·2	9·5	44·1	41·7 –47·7	71·8	68·9 –75·3
Nitrogen	·13	·05– ·20	·27	·14	·28	·34	·30	1·25	1·1 – 1·4	1·9	1·6 – 2·2
K_2O soluble in	·18	·12– ·34	·80	·18	·89	·72	·98	·47	·31– ·60	·24	·22– ·27
P_2O_5 strong HCl	·06	·05– ·12	·11	·08	·115	·19	·11	·195	·14– ·29	·15	·15– ·151
Subsoils											
Fine gravel	1·3	·0 – 6·6	·0	1·0	2·0	·7	·5	—	—	—	—
Coarse sand	81·8	66·0 –95·6	5·6	49·5	13·5	1·0	2·0	—	—	—	—
Fine sand	9·6	1·2 –19·5	10·1	44·1	19·5	10·0	2·9	—	—	—	—
Silt	1·8	·1 – 8·1	22·4	·5	22·5	32·4	12·7	—	—	—	—
Fine silt	1·2	·2 – 3·8	34·1	1·1	25·8	40·5	50·8	—	—	—	—
Clay	·3	·0 – 1·6	17·6	·0	5·2	4·7	19·5	—	—	—	—
Moisture	·3	·15– ·80	2·7	·25	4·3	2·2	3·1	4·6	2·12– 8·5	12·4	12·0 –12·8
Organic matter	1·4	·8 – 2·7	5·2	1·8	9·9	7·7	6·9	22·6	17·8 –30·9	79·5	79·0 –79·9

by the seventh value from the top of the list. If the number had been 28, the quartiles would have been taken as midway between the seventh and eighth values from the bottom and top respectively. In the case in point the quartiles are 18·4 and 22·6 respectively. The interquartile range is 22·6 − 18·4 = 4·2. In the table, the limits are given under the heading interquartile range and indicate the extremes between which half of the results lie. Interquartile ranges are given for the Anglesey medium loam and the Palæozoic silt loam only, as in the other cases not enough analyses are available to give any meaning to this or any other measure of dispersion. One point remains to be noticed. In a series of results lying between two limits, if the distribution is perfectly even, the interquartile range will be, *ex hypothesi*, half the range. Where there is a tendency for the results to approximate to an average value the interquartile range will be less than half the range. It will be seen on reference to the table that this is markedly the case in those soil types for which interquartile ranges are given, the interquartile range being generally about one-third of the range.

1. *Anglesey Medium Loam.*

This soil type occurs over the greater part of Anglesey. It is most commonly the result of the weathering of schistose materials of Pre-Cambrian age either *in situ* or in drift deposits. Soils formed from Granite, Ordovician grits and Old Red Sandstone are so similar in properties and composition to the soils formed from schists that they are, for the present, classed along with them.

The schists, from which the majority of these rocks are formed, are an exceedingly interesting but complicated series of rocks. They are of course metamorphic. The original materials from which they have been formed are of two classes, namely igneous and sedimentary. It has not been possible up to the present to correlate these rock types with any distinctions in soil properties at present. Generally speaking these Pre-Cambrian rocks are dark green, finely crystalline rocks, often highly contorted and in many places containing quartz veins.

In texture these soils are medium loams, often rather stony, of considerable fertility. In colour ("*nimium ne crede colori*") they vary from chocolate to fairly light brown. The subsoils are much lighter in colour and are often bright yellow. The depth is generally satisfactory and the natural drainage is good in the case of sedentary soils. The drift soils, generally in hollows, are not so well drained and, unless

artificial drainage has been carried out, bear a herbage of coarse grass and rushes.

The Anglesey medium loams are fairly well cultivated. Patches of waste occur at intervals where the rock rises above the surface and the surrounding soil is too shallow for cultivation. The most usual rotation is grass, cut for hay for two years and grazed for two, three or more years, oats, roots and oats. Comparatively little barley and wheat are grown. The district is chiefly noted for its black cattle.

The country is generally rather bleak and wind-swept. If the force of the wind were broken by shelter-belts, the agriculture of this area might compare with that of the best districts of England. Market gardening, in view of the mildness of the climate and the suitability of the soil, seems to offer a good prospect. Bulb-growing has been very successfully practised in places.

The figures in the table are the average of twenty-six samples. As will be seen the various fractions are fairly well balanced, no fraction being markedly predominant. Calcium carbonate was only found in one sample. The subsoils approximate very closely to the soils in mechanical composition.

Two sub-types must be noticed. There is a sandy type occurring along the south-western coast of Anglesey, which is formed by the admixture of blown sand with soil of the chief type. All gradations can be found from open dune to ordinary loam unmixed with sand. Except in the extreme south, where market gardening is practised on the sandy soils of Newborough, the ordinary type of Anglesey agriculture persists up to the fringe of the areas of sandy waste. Five samples were examined and average figures are shown in the table. Calcium carbonate was always absent. It is noteworthy that although very sandy these soils contain almost as much potash as the soils of the main type.

Another sub-type which has only been separated from the main type on account of a difference in chemical composition is that found as a result of the disintegration of granite. It resembles the main type in all respects except that it contains a much higher proportion of potash. As only two soils of this type have been examined the sub-division is unimportant, but the average is given in the table. It will be noticed that the potash figures lie well outside the range of the main type.

The soils formed from drift deposits are slightly heavier than the sedentary soils but the difference is not sufficiently marked to separate them from the main type.

TABLE II. *Anglesey Medium Loam.*

	Llandegfan 1		Cremlyn 5		Amlwch 6		Lasdre 7		Hologwyn 8		Llanddona 9	
	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil
Fine gravel	14·24	15·55	11·45	15·90	1·88	4·30	8·55	10·20	9·27	12·54	7·63	15·74
Coarse sand	21·50	17·64	19·31	18·15	11·38	10·95	12·32	9·59	17·59	20·12	19·90	21·75
Fine sand	15·97	23·65	17·54	16·41	29·17	32·22	20·61	27·96	17·66	16·57	20·35	22·67
Silt	9·46	13·06	10·02	10·06	13·99	15·49	15·05	16·75	15·38	14·48	14·21	12·60
Fine silt	17·67	15·84	18·60	18·96	18·83	18·69	23·68	21·27	18·52	16·34	19·01	13·79
Clay	4·50	4·01	5·90	7·95	3·70	7·19	4·79	3·82	6·52	7·45	4·97	5·64
Moisture	4·92	3·24	4·08	3·48	6·48	3·24	4·60	3·28	5·18	4·96	3·58	2·26
Organic matter	8·72	5·82	9·10	6·54	10·28	5·98	9·42	6·22	8·08	5·56	7·74	4·88
Calcium carbonate ...	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Nitrogen	·294	—	·288	—	·343	—	·336	—	·260	—	·281	—
48 hours' digestion with HCl:												
Potash (K_2O)	·610	—	·540	—	·476	—	·472	—	·452	—	·591	—
Phosphoric acid (P_2O_5) ...	·190	—	·213	—	·151	—	·158	—	·107	—	·142	—
Calcium oxide (CaO) ...	·38	—	·33	—	·36	—	·30	—	·73	—	·47	—
Magnesium oxide (MgO)...	·68	—	·80	—	·33	—	·14	—	—	—	—	—
Insoluble	73·9	—	72·7	—	74·1	—	74·8	—	74·5	—	76·7	—
Soluble in 1 % citric:												
Potash (K_2O)	·015	—	·013	—	·013	—	·018	—	·013	—	·016	—
Phosphoric acid (P_2O_5) ...	·009	—	·019	—	·010	—	·012	—	·011	—	·009	—

TABLE II (*continued*). *Anglesey Medium Loam.*

	Trefengan A 11		Henllys Fawr, Ty Croes 16		Bodedern 21		Cafnan 22		Gwredog Rhosgoch 23		Treasseth, Llangaffo 25	
	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil
Fine gravel ...	5·08	6·94	8·47	7·03	10·71	9·56	5·02	8·30	11·06	15·31	7·21	10·00
Coarse sand ...	27·54	28·46	18·46	15·46	18·51	13·50	18·98	13·71	9·72	12·57	18·47	19·86
Fine sand ...	23·06	22·66	22·57	24·58	16·71	18·78	22·90	24·26	20·62	29·23	21·48	19·90
Silt ...	10·85	11·45	16·06	17·63	15·21	20·70	11·55	13·26	14·77	10·93	13·68	11·90
Fine silt ...	15·54	15·91	16·07	16·71	21·56	20·72	20·95	21·74	21·94	21·43	18·16	18·63
Clay ...	4·35	5·37	6·57	9·37	4·04	6·22	7·55	5·23	4·74	8·30	5·80	7·59
Moisture ...	2·84	2·28	3·34	3·14	2·29	2·38	3·42	2·46	4·00	3·84	3·98	3·64
Organic matter ...	6·96	5·12	6·80	5·12	9·14	6·20	9·00	6·18	10·98	7·54	8·28	6·50
Calcium carbonate ...	Nil	Nil	Nil	Nil	·06	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Nitrogen ...	·227	—	·231	—	·312	—	·312	—	·358	—	·294	—
48 hours' digestion with HCl:												
Potash (K_2O) ...	·461	—	·530	—	·491	—	·476	—	·476	—	·521	—
Phosphoric acid (P_2O_5) ...	·112	—	·145	—	·049	—	·166	—	·277	—	·346	—
Calcium oxide (CaO) ...	·34	—	·31	—	·63	—	·32	—	·40	—	·41	—
Magnesium oxide (MgO) ...	·33	—	·30	—	·13	—	·21	—	·24	—	·33	—
Insoluble ...	81·0	—	78·8	—	75·9	—	76·2	—	70·2	—	76·1	—
Soluble in 1 % citric:												
Potash (K_2O) ...	·021	—	·014	—	·009	—	·020	—	·012	—	·019	—
Phosphoric acid (P_2O_5) ...	·009	—	·016	—	·006	—	·010	—	·022	—	·079	—

TABLE II (*continued*). *Anglesey Medium Loam.*

	Rhoscolyn 24		Baron Hill 29		Llangaffo 32		Llanfachreth 47		Llanddeusant 48		Holyhead 56	
	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil
Fine gravel ...	4·22	6·67	12·07	13·69	4·34	6·35	12·00	14·23	8·11	13·00	10·77	12·00
Coarse sand ...	29·17	27·42	26·41	23·36	22·01	22·90	16·39	14·42	10·73	13·05	21·81	17·92
Fine sand ...	26·70	23·33	21·04	22·24	23·79	24·90	16·08	21·40	20·87	21·94	18·41	21·21
Silt ...	15·91	14·82	9·36	12·83	12·12	14·39	17·00	14·12	12·57	15·11	14·96	17·08
Fine silt ...	13·18	14·05	14·79	11·20	21·92	16·37	20·55	20·67	24·25	18·68	17·10	16·73
Clay ...	1·92	2·50	3·62	4·43	4·94	7·47	3·70	5·16	8·55	8·79	2·50	3·09
Moisture ...	2·80	2·26	2·72	1·74	2·60	1·86	1·58	1·66	3·54	2·22	2·82	2·32
Organic matter ...	7·58	6·60	8·16	5·88	7·98	4·64	9·02	6·62	9·30	6·35	5·02	3·98
Calcium carbonate ...	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	1·52	·30
Nitrogen ...	·264	—	·244	—	·267	—	·287	—	·305	—	·158	—
48 hours' digestion with HCl :												
Potash (K_2O) ...	·340	—	·483	—	·471	—	·583	—	·660	—	·614	—
Phosphoric acid (P_2O_5) ...	·111	—	·138	—	·095	—	·162	—	·178	—	·150	—
Calcium oxide (CaO) ...	·51	—	·32	—	·41	—	·33	—	·58	—	—	—
Magnesium oxide (MgO) ...	·21	—	1·01	—	·42	—	·28	—	·20	—	—	—
Insoluble	78·9	—	75·2	—	78·3	—	75·4	—	72·5	—	76·2	—
Soluble in 1 % citric :												
Potash (K_2O) ...	·021	—	·016	—	·019	—	·011	—	·014	—	·024	—
Phosphoric acid (P_2O_5) ...	·006	—	·030	—	·013	—	·011	—	·015	—	·019	—

TABLE II (*continued*).   *Anglesey Medium Loam.*

	Llangefni 13		Cwyrtai 15		Paradwys 14		Llwyn-On 28	
	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil
Fine gravel	4·58	7·27	4·09	6·69	7·36	7·05	10·63	9·11
Coarse sand	12·16	10·68	7·66	8·72	23·92	24·08	16·53	17·98
Fine sand	23·67	24·48	20·01	21·13	19·90	20·00	18·41	17·92
Silt	17·03	21·80	16·41	16·88	12·02	14·75	15·11	17·07
Fine silt	20·48	17·08	23·12	19·43	16·58	17·48	17·34	16·50
Clay	5·68	10·24	9·42	13·71	4·76	6·52	6·03	10·87
Moisture	6·06	2·32	6·22	5·96	5·18	2·82	3·54	2·14
Organic matter	9·20	4·52	10·08	5·14	7·98	5·68	10·20	4·70
Calcium carbonate	Nil	Nil	Nil	Nil	Nil	·17	Nil	Nil
Nitrogen	·291	—	·330	—	·263	—	·281	—
48 hours' digestion with HCl ·								
Potash (K₂O)	·425	—	·515	—	·530	—	·580	—
Phosphoric acid (P₂O₅) ...	·098	—	·100	—	·145	—	·134	—
Calcium oxide (CaO) ...	·28	—	·33	—	·35	—	·87	—
Magnesium oxide (MgO) ...	·28	—	·40	—	·42	—	·13	—
Insoluble	75·0	—	72·6	—	76·5	—	72·1	—
Soluble in 1 % citric :								
Potash (K₂O)	·022	—	·013	—	·014	—	·013	—
Phosphoric acid (P₂O₅) ...	·013	—	·014	—	·016	—	·020	—

	Bodfeddan 18		Cefn-Poeth 26		Plascylched 30		Bodafon 20	
	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil
Fine gravel	10·63	15·42	10·99	13·55	7·55	12·93	10·73	18·58
Coarse sand	15·42	17·20	14·80	18·95	23·36	21·88	18·09	14·94
Fine sand	20·40	19·46	23·29	18·89	21·80	20·67	26·72	23·43
Silt	13·78	16·50	10·71	14·99	13·25	15·50	13·45	14·10
Fine silt	16·53	15·94	16·22	16·09	18·23	16·58	15·40	15·31
Clay	4·36	4·34	4·76	6·05	3·82	3·72	3·80	5·29
Moisture	4·20	3·68	5·00	1·68	1·48	·80	2·78	2·04
Organic matter	9·54	6·22	11·60	7·54	8·00	3·83	8·24	5·34
Calcium carbonate	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Nitrogen	·336	—	·337	—	·280	—	·279	—
48 hours' digestion with HCl ·								
Potash (K₂O)	·443	—	·496	—	·510	—	·590	—
Phosphoric acid (P₂O₅) ...	·141	—	·184	—	·086	—	·179	—
Calcium oxide (CaO) ...	·45	—	·28	—	·44	—	·29	—
Magnesium oxide (MgO) ...	·29	—	·17	—	·60	—	·20	—
Insoluble	75·2	—	66·1	—	78·7	—	77·5	—
Soluble in 1 % citric :								
Potash (K₂O)	·021	—	·020	—	·014	—	·019	—
Phosphoric acid (P₂O₅) ...	·012	—	·009	—	·016	—	·012	—

TABLE II (continued). *Anglesey Medium Loam.*

| | Sub-type A | | | | | | | | | | Sub-type B | | | |
| | Penseri. Ty Groes | | Trefry 49 | | Cae Coch. Newborough 52 | | Cellar, Aberffraw 58 | | Cellar, Aberffraw 58 A | | Sarn Fadog 55 | | Bod Suran 53 | |
	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil
Fine gravel	7·69	9·58	4·69	2·46	3·39	7·00	4·67	5·35	5·23	—	6·41	12·28	10·97	13·75
Coarse sand	43·98	31·00	66·64	72·05	38·63	28·90	50·07	43·68	43·68	—	10·63	12·76	15·79	23·37
Fine sand	14·38	19·85	10·87	10·96	20·04	22·47	16·96	20·09	16·65	—	21·81	18·09	20·53	19·11
Silt	9·77	9·17	5·52	6·25	12·00	14·34	11·02	11·84	8·73	—	13·11	10·66	13·43	15·38
Fine silt	11·43	13·99	5·79	3·22	12·50	13·55	6·62	8·60	13·37	—	21·71	17·04	17·42	10·99
Clay	2·88	5·46	1·23	1·43	2·82	3·20	2·89	3·42	3·30	—	10·08	8·49	5·29	4·55
Moisture	3·02	2·64	1·04	·84	1·44	1·36	1·96	1·46	1·97	—	4·42	2·10	4·36	2·22
Organic matter	5·80	4·72	4·18	2·66	6·30	4·66	5·52	2·70	5·36	—	9·80	6·90	10·46	7·38
Calcium carbonate	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	—	Nil	Nil	Nil	Nil
Nitrogen	·209	—	·160	—	·204	—	·238	—	—	—	·310	—	·310	—
48 hours' digestion with HCl:														
Potash (K_2O)	·388	—	·628	—	·580	—	·370	—	—	—	·696	—	·880	—
Phosphoric acid (P_2O_5)	·113	—	·184	—	·105	—	·099	—	—	—	·172	—	·210	—
Calcium oxide (CaO)	·31	—	·21	—	—	—	—	—	—	—	—	—	—	—
Magnesium oxide (MgO)	·30	—	·23	—	—	—	—	—	—	—	—	—	—	—
Insoluble	83·5	—	88·2	—	82·1	—	84·6	—	—	—	71·0	—	71·2	—
Soluble in 1 % citric:														
Potash (K_2O)	·018	—	·006	—	·012	—	·016	—	—	—	·013	—	·024	—
Phosphoric acid (P_2O_5)	·004	—	·012	—	·009	—	·007	—	—	—	·005	—	·006	—

Palæozoic Silt Loam.

This is the best defined and the most widely spread soil type hitherto encountered in North Wales. It covers practically the whole of Denbighshire and occurs to a more limited extent in Central Anglesey and parts of South Carnarvonshire. Outside of the district investigated in the present paper it covers a considerable area. It extends southward over a large part of Merionethshire and through South-west Shropshire and Montgomeryshire into South and Central Wales, where it probably attains a very large extent. The Palæozoic silt loam is therefore one of the most important soil types hitherto encountered.

These soils are derived from the weathering, generally *in situ*, of the sedimentary rocks of the Cambrian, Ordovician and Silurian formations. The rocks in question form an enormous succession of shales, mudstones, flags and grits. It is much to be regretted that petrologists have so neglected the sedimentary rocks, particularly the older shales and flagstones. Practically no literature is available on the petrology of the North Welsh Palæozoic sediments although the igneous rocks have been exhaustively studied by Harker, Teall and others. Lithologically these rocks vary from extremely soft shales to hard flagstones such as are found in the Cambrian area around Portmadoc. All the rocks can however be cut with an ordinary knife. The slates of North Wales are of course metamorphosed sediments, but a considerable degree of metamorphosis has occurred in the rocks not usually thus regarded, for many of them are sub-crystalline.

Associated limestones are almost lacking in North Wales, although eastward in Shropshire and Staffordshire large masses of limestone occur in the Silurian formation. It is almost certain that the shore-line at the time of deposition lay westward, in the direction of the Irish Sea.

In North Wales the Palæozoic silt loam is fairly uniform in character, the variations being due generally to water content and situation. Typically, it is a rather stony medium loam of variable but generally satisfactory depth. The colour varies from grey to brown in the soil; the subsoil is often yellow or light brown and contains high proportions of stones and gravel from the underlying rock. Most of the soil up to about 1000 feet is fairly well drained naturally but at higher elevations the land is often wet. A large part of the central uplands of Denbighshire consists of stretches of rather wet heather moor with accumulations of peat in the depressions.

The agricultural value of these soils varies according to climate and situation. In sheltered places the standard of cultivation is fairly high. In the counties of Shropshire and Montgomery where considerable areas of these soils occur in sheltered valleys, the farming is very good. This is also true of the outlying areas in Anglesey and Carnarvonshire.

In Denbighshire, where the Palæozoic silt loam is typically developed, and where it forms so large a part of the total area, the farming may be described as good second-rate cultivation. The rotation of crops is similar to that practised in Anglesey. Denbighshire is largely a county of sheep-farming: the upland moors provide abundant rough grazing in the summer months. While the Anglesey medium loam would appear to be suitable for further development in the direction of market gardening, it cannot be said that this type of soil as developed in Denbighshire is likely to be suitable for a more intensive system of husbandry, although there is room for much improvement along the lines of existing practices.

The Palæozoic silt loam of Anglesey shows very little difference from the rest of Anglesey in the agriculture practised on it, except that the grass land is possibly a little better than on the Anglesey medium loam.

The average composition of forty-nine samples is shown in the general table. As will be seen from the figures for range and interquartile range these soils form a very well-defined type. The most noteworthy feature is that the fine silt is the dominant fraction, being on the average nearly 29 % of the soil. The percentage of clay is only 6·6. The consequence of this is that these soils are very sticky when wet but, owing to the comparatively small amount of clay and, possibly, to the high proportions of gravel and organic matter, are fairly friable on drying. A number of determinations of plasticity were made according to the method of Atterberg[1]. The largest plasticity number obtained was 10 for a Carnarvonshire soil containing 30·5 % fine silt and 12·35 % clay. This may be compared with the figure 23·9 obtained for a glacial clay of the vale of Clwyd. In order to determine the effect of fine silt on the plasticity of a soil, the clay was removed by sedimentation from a soil containing 34·4 % of fine silt and 14·9 % clay. The residual material was found to have entirely lost its plasticity, i.e. it could only be rolled without disintegration when it contained so much water as to render it capable of behaving as a fluid.

Chemically these soils are notable for their high proportions of potash soluble in hydrochloric acid—·80 % on an average. Phosphoric

[1] *Int. Mitt. für Bodenkunde*, vol. I, p. 10.

TABLE III. *Palæozoic Silt Loam. Anglesey (Ordovician).*

	Gwredog, Amlwch 42		Llwydiarth Esgob 43		Llwydiarth Esgob 44		Gwredog. Ll-medd 45		Bodsuran 54		Abererch 60	
	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil
Fine gravel ...	14·14	23·67	5·98	5·89	7·76	6·30	4·08	4·26	7·29	12·22	5·86	7·09
Coarse sand ...	8·47	9·22	9·85	12·51	11·94	10·62	7·09	5·45	10·68	15·72	12·08	11·94
Fine sand ...	22·33	25·14	19·48	19·46	14·83	14·60	13·33	11·27	17·90	14·80	16·98	17·31
Silt ...	6·88	6·95	15·04	17·02	11·23	14·22	14·08	14·14	12·47	17·35	13·68	14·85
Fine silt ...	20·71	23·80	26·79	21·15	26·43	26·84	30·48	29·70	25·64	19·95	30·01	30·20
Clay ...	6·72	6·58	10·79	13·47	11·65	15·26	14·16	19·05	7·98	7·13	7·47	6·38
Moisture ...	3·26	2·16	2·34	2·00	2·64	2·56	2·76	2·78	4·00	2·84	2·32	1·84
Organic matter ...	14·40	9·76	8·96	6·36	10·44	7·56	9·84	8·90	11·98	7·78	10·40	8·40
Calcium carbonate ...	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Nitrogen ...	·454	—	·276	—	·327	—	·406	—	·366	—	·356	—
48 hours' digestion with HCl :												
Potash (K_2O) ...	·646	—	·750	—	·851	—	·850	—	·865	—	·824	—
Phosphoric acid (P_2O_5) ...	·267	—	·120	—	·188	—	·195	—	·210	—	·145	—
Calcium oxide (CaO) ...	·50	—	·34	—	·30	—	·28	—	—	—	—	—
Magnesium oxide (MgO)...	·32	—	·28	—	·20	—	·30	—	—	—	—	—
Insoluble ...	65·3	—	71·9	—	68·2	—	64·1	—	66·8	—	75·4	—
Soluble in 1 % citric :												
Potash (K_2O) ...	·020	—	·018	—	·018	—	·014	—	·010	—	·015	—
Phosphoric acid (P_2O_5) ...	·012	—	·017	—	·010	—	·013	—	·004	—	·010	—

TABLE III (continued). Palaeozoic Silt Loam. Denbighshire (Silurian).

	Anglesey (Ordovician) Abererch A61		Voel Ganol D36		Fachlwyd Uchaf D37		Berwyn D38		Llanynys D39		Nant melai D41	
	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil
Fine gravel ...	8·79	9·00	15·96	16·24	16·11	—	13·17	12·78	12·32	22·21	4·05	8·63
Coarse sand ...	12·44	11·16	3·18	5·98	5·36	—	4·86	4·12	9·78	9·72	3·33	4·45
Fine sand ...	20·33	15·45	10·59	11·51	8·93	—	11·33	13·97	10·62	14·33	7·31	7·16
Silt ...	14·72	13·97	21·16	24·88	18·01	—	21·00	25·16	18·13	18·75	19·10	19·04
Fine silt ...	24·33	23·24	27·93	25·38	26·94	—	26·51	27·31	22·83	22·34	39·27	35·92
Clay ...	6·34	6·23	5·67	6·48	6·48	—	4·73	4·00	7·22	4·35	8·23	8·80
Moisture ...	2·26	1·58	1·66	2·20	2·04	—	2·93	2·55	4·80	1·14	2·39	2·51
Organic matter ...	9·52	8·18	11·44	6·80	14·50	—	13·90	9·51	9·72	5·42	12·75	10·83
Calcium carbonate ...	Nil	Nil	Nil	Nil	Nil	—	Nil	Nil	·93	·65	Nil	Nil
Nitrogen ...	·350	—	·340	—	·344	—	·610	—	·380	—	·470	—
48 hours' digestion with HCl:												
Potash (K₂O) ...	·782	—	·700	—	·850	—	·803	—	·850	—	·800	—
Phosphoric acid (P₂O₅) ...	·140	—	·124	—	·175	—	·164	—	·153	—	·132	—
Calcium oxide (CaO) ...	—	—	—	—	—	—	—	—	—	—	—	—
Magnesium oxide (MgO) ...	—	—	—	—	—	—	—	—	—	—	—	—
Insoluble ...	77·3	—	73·0	—	68·4	—	67·2	—	70·1	—	70·0	—
Soluble in 1 % citric:												
Potash (K₂O) ...	·016	—	·017	—	·014	—	·017	—	·023	—	·022	—
Phosphoric acid (P₂O₅) ...	008	—	004	—	005	—	·018	—	·011	—	·009	—

TABLE III (*continued*). *Palæozoic Silt Loam. Denbighshire (Silurian).*

	Henllan D42		(Ordovician) Cerryg-y-drudion D43		Llansannan D44		Pantyrhedyn D46		Pantyrhedyn D45		Chirk D50	
	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil
Fine gravel ...	6·08	6·10	3·54	7·80	6·79	—	9·02	10·03	10·34	2·83	10·55	18·10
Coarse sand ...	10·03	10·35	3·71	5·31	3·80	—	2·17	3·14	2·77	1·96	8·13	7·43
Fine sand ...	13·20	13·37	12·16	15·50	15·03	—	14·81	12·25	19·17	20·82	16·53	15·82
Silt ...	19·27	22·85	11·09	15·85	15·84	—	13·51	22·14	14·94	22·44	17·20	18·89
Fine silt ...	32·67	30·77	34·90	31·84	38·08	—	32·02	29·26	30·22	31·10	27·04	24·85
Clay ...	5·37	8·35	7·10	12·88	5·90	—	8·18	9·17	6·23	8·54	4·26	4·96
Moisture ...	1·46	1·12	3·81	1·80	2·24	—	3·46	2·52	3·30	2·22	2·92	—
Organic matter ...	7·34	4·94	23·24	9·65	10·94	—	14·38	7·66	12·60	8·22	13·58	—
Calcium carbonate ...	1·24	·25	Nil	Nil	Nil	—	Nil	Nil	Nil	Nil·	Nil	—
Nitrogen ...	·298	—	·683	—	·386	—	·392	—	·456	—	·549	—
48 hours' digestion with HCl :												
Potash (K$_2$O) ...	·650	—	·646	—	·678	—	·675	—	·600	—	1·070	—
Phosphoric acid (P$_2$O$_5$) ...	·092	—	·255	—	·123	—	·111	—	·165	—	·195	—
Calcium oxide (CaO) ...	—	—	—	—	—	—	—	—	—	—	—	—
Magnesium oxide (MgO) ...	—	—	—	—	—	—	—	—	—	—	—	—
Insoluble ...	76·0	—	65·9	—	72·6	—	74·34	—	72·49	—	74·4	—
Soluble in 1 % citric :												
Potash (K$_2$O) ...	·018	—	·025	—	·024	—	·020	—	·017	—	—	—
Phosphoric acid (P$_2$O$_5$) ...	·005	—	·048	—	·008	—	·016	—	·018	—	—	—

TABLE III (*continued*). *Palæozoic Silt Loam. Denbighshire (Silurian)*.

	Llanrwst D 52		Llanelidan D 55		Glanconwy D 57	
	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil
Fine gravel	16·48	—	26·11	28·79	13·46	20·93
Coarse sand	12·71	—	7·92	9·32	7·69	9·38
Fine sand	12·44	—	8·25	8·27	11·83	9·05
Silt	13·49	—	10·43	10·60	17·94	20·00
Fine silt	25·67	—	29·04	25·26	27·72	22·46
Clay	3·72	—	5·78	6·38	7·20	7·29
Moisture	1·84	—	1·50	2·36	2·18	1·80
Organic matter	10·46	—	12·84	8·86	9·14	6·28
Calcium carbonate	Nil	—	Nil	—	Nil	Nil
Nitrogen	·408	—	·468	—	·304	—
48 hours' digestion with HCl :						
Potash (K_2O)	·986	—	1·171	—	·873	—
Phosphoric acid (P_2O_5) ...	·205	—	·205	—	·138	—
Calcium oxide (CaO) ...	—	—	—	—	—	—
Magnesium oxide (MgO)...	—	—	—	—	—	—
Insoluble	67·86	—	67·72	—	69·29	—
Soluble in 1 % citric :						
Potash (K_2O)	·027	—	·024	—	·021	—
Phosphoric acid (P_2O_5) ...	·010	—	·007	—	·007	—

TABLE III (continued). *Palæozoic Silt Loam. Denbighshire (Silurian).*

	Nilig Hiraethog D18		Clawddnewydd D20		Voel Isaf, Groes, Denbigh D22		Nantglyn D23		Llanarmon-Dyffryn-Ceiriog D24		Llangerniew D28	
	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil
Fine gravel	7·31	11·64	11·67	17·59	12·80	18·91	3·49	10·84	8·24	6·60	13·16	26·17
Coarse sand	7·44	11·60	6·53	7·74	5·16	8·04	2·67	6·35	6·45	6·03	9·79	·9·70
Fine sand	17·99	10·35	12·54	12·64	10·80	13·00	8·15	9·41	22·08	9·85	17·10	9·95
Silt	16·79	18·64	14·92	14·59	15·73	16·62	19·58	17·09	16·06	27·49	9·57	17·42
Fine silt	25·27	25·76	29·91	25·06	32·50	18·55	40·83	34·96	21·58	27·08	28·64	22·43
Clay	7·20	7·81	5·99	6·64	5·15	4·20	5·80	7·81	9·26	13·69	7·93	3·89
Moisture	2·86	2·86	2·98	3·10	3·66	4·26	6·18	1·82	3·08	2·10	2·36	2·06
Organic matter	11·96	8·54	11·82	7·80	13·52	10·48	12·48	7·80	9·50	5·94	9·36	8·14
Calcium carbonate ...	Nil	Nil	·22	·19	Nil	·Nil	Nil	Nil	Nil	Nil	Nil	Nil
Nitrogen	·420	—	·460	—	·392	—	·410	—	·316	—	·367	—
48 hours' digestion with HCl :												
Potash (K₂O)	·788	—	·750	—	·614	—	·740	—	·690	—	·730	—
Phosphoric acid (P₂O₅) ...	·202	—	·250	—	·193	—	·142	—	·132	—	·212	—
Calcium oxide (CaO) ...	—	—	—	—	—	—	—	—	—	—	—	—
Magnesium oxide (MgO)...	—	—	—	—	—	—	—	—	—	—	—	—
Insoluble	69·9	—	70·1	—	69·2	—	68·9	—	73·2	—	70·9	—
Soluble in 1 % citric :												
Potash (K₂O)	—	—	·021	—	·027	—	—	—	—	—	·019	—
Phosphoric acid (P₂O₅) ...	—	—	·007	—	·011	—	—	—	—	—	·021	—

TABLE III (continued). Palæozoic Silt Loam. Denbighshire (Silurian).

	Llanfairtalhaiarn D30		Gyffylliog D33		Maes Mynan, Caerwys D1		Pentrellyn cymmer. Cerryg D9		Llansannan D15		Gyffylliog D16	
	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil
Fine gravel	13·30	35·46	6·19	15·37	15·80	15·70	16·34	18·77	6·01	9·52	12·90	—
Coarse sand	6·47	6·09	4·84	8·84	11·00	15·53	11·20	10·24	5·52	6·24	11·84	—
Fine sand	11·96	9·05	10·38	4·91	11·22	10·29	14·40	15·83	24·01	30·60	14·63	—
Silt	20·89	25·98	19·23	16·68	13·34	18·27	9·64	16·08	8·45	11·33	11·15	—
Fine silt	26·66	12·92	31·97	27·00	26·62	21·21	26·23	23·62	24·26	23·92	29·54	—
Clay	4·11	3·08	7·15	8·28	5·41	4·51	4·20	7·87	5·62	6·06	4·46	—
Moisture	3·44	1·44	2·21	1·20	4·05	3·64	2·34	1·50	3·04	2·44	2·74	1·44
Organic matter	11·14	5·18	15·15	7·68	10·66	6·70	12·70	7·18	11·28	6·96	11·18	5·74
Calcium carbonate	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	·21	·30
Nitrogen	·400	—	·490	—	·301	—	·430	—	·416	—	·396	—
48 hours' digestion with HCl:												
Potash (K$_2$O)	·682	—	·846	—	·850	—	·535	—	1·048	—	·527	—
Phosphoric acid (P$_2$O$_5$)	·201	—	·117	—	·144	—	·325	—	·205	—	·287	—
Calcium oxide (CaO)	—	—	—	—	·20	—	·60	—	—	—	—	—
Magnesium oxide (MgO)	—	—	—	—	·37	—	·30	—	—	—	—	—
Insoluble	68·6	—	67·9	—	70·0	—	68·7	—	70·0	—	68·0	—
Soluble in 1 % citric:												
Potash (K$_2$O)	·030	—	·023	—	·031	—	·014	—	·030	—	·022	—
Phosphoric acid (P$_2$O$_5$)	·009	—	·0095	—	·007	—	·012	—	·007	—	·014	—

TABLE III (*continued*). *Palæozoic Silt Loam. Denbighshire (Silurian).*

	Llanefydd D 17		Nilig D 34		Caegwyn D 35		Llanelidan D 56	
	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil
Fine gravel	9·00	17·22	·50	·63	3·55	5·97	2·90	—
Coarse sand	7·87	10·60	2·04	2·39	2·56	3·89	3·56	—
Fine sand	13·94	12·67	9·15	9·91	13·31	7·39	13·85	—
Silt	13·99	25·14	23·17	22·25	15·57	32·42	20·59	—
Fine silt	35·23	18·46	38·33	38·74	36·29	23·79	38·97	—
Clay	5·62	4·68	11·44	17·05	9·93	14·22	7·30	—
Moisture	2·64	1·62	1·58	1·25	2·15	1·22	·86	—
Organic matter	10·40	6·56	10·72	6·66	14·94	8·20	10·08	—
Calcium carbonate	Nil	Nil	Nil	Nil	Nil	Nil	Nil	—
Nitrogen	·380	—	·450	—	·447	—	·350	—
48 hours' digestion with HCl :								
Potash (K_2O)	·966	—	1·203	—	·803	—	·890	—
Phosphoric acid (P_2O_5) ...	·186	—	·057	—	·109	—	·125	—
Calcium oxide (CaO) ...	—	—	—	—	—	—	—	—
Magnesium oxide (MgO)...	—	—	—	—	—	—	—	—
Insoluble	70·3	—	73·2	—	69·6	—	72·5	—
Soluble in 1 °/o *citric :*								
Potash (K_2O)	—	—	·022	—	·017	—	·028	—
Phosphoric acid (P_2O_5) ...	—	—	·008	—	·009	—	·014	—

TABLE III (continued). *Palæozoic Silt Loam. Denbighshire (Ordovician).*

	Llanarmon-Dyffryn-Ceiriog D53		Llanarmon-Dyffryn-Ceiriog D54		Llangedwyn D2		Llangedwyn D3		Llansilin D4		Llanfihangel D2	
	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil
Fine gravel ...	13·67	22·53	21·09	23·50	18·82	23·35	5·37	4·50	10·36	25·66	12·36	16·97
Coarse sand ...	5·81	7·07	8·53	8·22	10·57	13·35	5·23	8·71	4·79	11·50	5·46	8·02
Fine sand ...	9·39	9·50	9·42	9·40	10·41	9·02	14·34	14·88	11·41	10·17	22·94	18·32
Silt ...	13·16	13·41	12·71	12·91	12·02	14·20	14·39	14·08	12·45	13·58	13·74	16·94
Fine silt ...	32·80	25·27	28·71	27·59	26·94	22·25	33·01	29·09	31·64	20·10	20·20	19·87
Clay ...	6·88	5·19	7·10	6·64	5·00	6·08	9·13	3·40	8·63	15·33	3·30	4·18
Moisture ...	1·94	—	·90	—	4·24	3·10	4·32	3·60	5·74	4·72	5·98	3·60
Organic matter ...	13·56	—	12·34	—	11·70	7·72	11·22	8·06	12·40	7·26	14·14	10·62
Calcium carbonate ...	Nil	—	Nil	—	·47	·19	Nil	Nil	·23	·11	Nil	Nil
Nitrogen ...	·451	—	·428	—	·357	—	·317	—	·256	—	·520	—
48 hours' digestion with HCl :												
Potash (K_2O) ...	·990	—	·822	—	·656	—	·671	—	·714	—	·740	—
Phosphoric acid (P_2O_5) ...	·232	—	·162	—	·263	—	·232	—	·252	—	·214	—
Calcium oxide (CaO) ...	—	—	—	—	·81	—	·55	—	·38	—	—	—
Magnesium oxide (MgO)...	—	—	—	—	·57	—	·42	—	·36	—	—	—
Insoluble ...	59·84	—	65·4	—	64·5	—	61·7	—	64·5	—	67·2	—
Soluble in 1 % citric :												
Potash (K_2O) ...	·023	—	·031	—	·024	—	·014	—	·024	—	·022	—
Phosphoric acid (P_2O_5) ...	·009	—	·017	—	·008	—	·009	—	·004	—	·013	—

TABLE III (continued). *Palæozoic Silt Loam. Carnarvonshire.*

	Cambrian				Ordovician						Silurian	
	Criccieth C12		Ynyscynhaiarn C14		Bachellyn C1		Penmachno C32		Penmachno C33		Maenan C48	
	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil
Fine gravel ...	14·92	15·96	14·46	18·20	4·66	8·72	8·50	18·44	6·89	3·78	8·52	25·23
Coarse sand ...	12·28	10·96	11·50	10·76	11·73	9·78	6·96	7·83	5·09	4·41	7·24	8·37
Fine sand ...	10·30	11·00	11·04	14·08	11·43	9·87	12·52	11·78	12·54	16·10	17·54	12·25
Silt ...	13·95	17·88	16·92	16·81	12·50	13·58	18·08	17·32	17·07	17·38	17·14	20·12
Fine silt ...	23·00	21·45	25·72	22·45	30·48	29·02	30·43	27·74	29·44	33·07	27·87	19·00
Clay ...	5·18	8·46	4·32	5·54	12·35	14·75	3·29	2·65	3·15	4·52	5·64	3·89
Moisture ...	4·54	3·20	3·94	2·06	4·68	4·36	3·28	3·08	4·26	3·48	3·12	2·80
Organic matter ...	13·34	9·28	12·22	7·02	9·76	8·38	15·56	10·88	19·96	15·64	11·00	8·88
Calcium carbonate ...	Nil	Nil	Nil	Nil	·09	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Nitrogen ...	·487	—	·440	—	·319	—	·566	—	·730	—	·372	—
48 hours' digestion with HCl :												
Potash (K₂O) ...	·512	—	·992	—	·649	—	·736	—	·570	—	·800	—
Phosphoric acid (P₂O₅) ...	·245	—	·206	—	·158	—	·150	—	·203	—	·294	—
Calcium oxide (CaO) ...	—	—	—	—	·28	—	—	—	—	—	—	—
Magnesium oxide (MgO) ...	—	—	—	—	·54	—	—	—	—	—	—	—
Insoluble ...	69·6	—	69·1	—	60·3	—	68·1	—	68·1	—	69·2	—
Soluble in 1 % citric :												
Potash (K₂O) ...	·030	—	·021	—	·052	—	·020	—	·050	—	—	—
Phosphoric acid (P₂O₅) ...	·030	—	·012	—	·014	—	·007	—	·021	—	—	—

acid is also present in fairly high proportion. Calcium carbonate, except in one or two samples, was always lacking.

In order to determine whether the soils formed from Ordovician material differed in any marked respect in composition from the main type the results of 15 Ordovician soils were averaged. The figures, however, except in the case of the fine gravel which was 2 % lower for the Ordovician soils, did not differ by more than 1 % from the average of the 49 samples of the series. Potash for the Ordovician soils was ·72 % as against ·80 % and phosphoric oxide was ·20 % as against ·185 %. It seems evident therefore that there is no reason for separating the Ordovician soils from the other soils of the series.

A comparison with the Palaeozoic silt loams of Shropshire is instructive. In that county the soils of Ordovician and Lower Silurian (Wenlock) age are considerably heavier than the Palaeozoic silt loams of North Wales and many of them are properly described as clay soils. The Upper Silurian (Ludlow) soils of Shropshire agree with the Palaeozoic silt loams of North Wales in containing comparatively little clay, but the dominant fraction is the silt instead of the fine silt. The rocks in the two areas appear similar but the Shropshire strata were certainly deposited in deeper water. Probably the original clay of the North Welsh rocks has been altered by metamorphosis whereas the clay of the Shropshire rocks may have survived from primitive times. The matter is of considerable interest and is worth investigation. It should be added that the Ordovician and Silurian soils of Shropshire are also rich in potash but do not show such high figures for phosphoric oxide. A large number of the Shropshire soils contain calcium carbonate.

Carnarvonshire Stony Loams.

These soils can scarcely be called a type. They form rather a series of soils, and occur in the parts of Carnarvonshire between the mountains, the northern coast and the line AB shown on the orographical map. In texture they are gravelly or stony medium loams sometimes varying a little on the light side and sometimes on the heavy side, but never becoming sands or clays. As a rule stones and boulders become more abundant as the mountains are approached. Geologically they consist of material scraped down from the mountain area by local glacial action. Generally the material is very much mixed but in certain places, a particular variety of rock material is seen to be dominant in the soil and can be referred to adjacent rocks in the mountain area. For example, in the valley immediately west of the summit of Snowdon, the stones

and gravel are almost entirely Cambrian shale. It is very exceptional in this area to find sedentary soils. Three soils have however been examined which appeared to be products of the decomposition *in situ* of the subjacent rock. These soils agriculturally and physically resembled other soils in the vicinity and were accordingly classed with them, although fuller work may place them in a separate class.

The agriculture of this district varies according to the altitude. In the lowlands the farming, as far as cultivation is concerned, resembles that in Anglesey, except that rather less cattle and more sheep are kept. In the uplands the holdings which are generally small are almost entirely in grass. Along the northern part of the area from Bangor to Conway this fringe of small grass holdings lying above the lowland farms is absent. Along the western foothills however there is an important belt of small grass farms. The small fields with their Cyclopean walls of lichen covered stones form a striking feature of the landscape. It is not uncommon to see fields of half an acre enclosed by massive walls five or six feet in height. When it is realised that the stones were removed from the land when it was reclaimed from the waste, a good idea is obtained of the nature of the soil of these foothills.

Since the soils of this category scarcely form a uniform type no useful purpose will be served by giving average analyses of all the soils examined. On inspecting the results, however, it appears that certain soils can be grouped together. Four sub-types have been thus obtained. A is the average of eight soils, mainly from South Carnarvonshire but including three from the north of the county. All the soils of this sub-type with one exception are from the best class of Carnarvonshire farms. B is the average of three soils apparently much less fertile than the A sub-type and lying at a greater altitude. C is the average of three sedentary soils in the Bangor district, one from quartz porphyry and the other two from a Pre-Cambrian metamorphic rock. These sedentary soils are equal in fertility to those of the A sub-type. D is the average of three soils from the poorly cultivated district lying due south of Carnarvon in the foothills of the central mountain system. The D soils are mainly composed of local igneous material. In A and B sedimentary material predominates.

The average analyses are shown in the general table.

No calcium carbonate is found in the soils of this series examined up to the present. The only points to be noted are the high proportions of fine gravel, particularly in sub-type C, the high potash and phosphoric acid figures in sub-type C, and the low potash figures in sub-type D.

TABLE IV. *Carnarvonshire Stony Loams.*

	Llanwnda C 36		Penbryn, Chwilog C 39		Penbryn, Chwilog C 40		Tynllanor C 41		Tynllanor C 42		Aber Ab. 2		Aber Ab. 7		Golan C 29	
	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil
Fine gravel ...	7·78	11·51	10·13	7·44	10·71	3·97	9·00	2·92	1·02	1·00	7·92	—	8·02	9·35	8·21	6·50
Coarse sand ...	12·32	13·02	9·94	9·45	7·75	9·50	8·56	7·79	4·07	5·72	17·59	—	9·23	7·88	5·77	4·90
Fine sand ...	20·48	18·42	22·07	13·06	21·05	13·91	24·21	12·60	23·53	20·53	17·61	—	20·56	25·50	23·20	12·79
Silt ...	15·58	14·29	10·87	22·87	13·10	28·88	12·35	25·68	18·55	21·10	12·44	—	20·41	32·42	11·17	23·56
Fine silt ...	23·40	23·24	26·37	26·01	24·94	22·96	24·22	27·79	23·15	25·16	20·50	—	23·95	11·31	25·82	24·54
Clay ...	5·64	8·69	5·00	5·37	4·80	6·91	6·39	10·20	7·16	11·03	4·10	—	2·67	3·38	5·88	8·69
Moisture ...	2·62	2·80	1·02	5·16	1·76	·94	3·30	2·66	4·12	2·52	2·7	1·6	5·08	2·56	4·74	5·06
Organic matter ...	10·34	7·56	14·00	9·16	17·28	8·52	12·08	8·02	17·52	11·34	10·4	6·4	7·81	6·32	14·38	11·56
Calcium carbonate ...	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Nitrogen ...	·470	—	·494	—	·462	—	·491	—	·593	—	·396	—	·254	—	·423	—
48 hours' digestion with HCl:																
Potash (K$_2$O) ...	·638	—	·656	—	·620	—	·680	—	·625	—	·461	—	·462	—	·585	—
Phosphoric acid (P$_2$O$_5$) ...	·110	—	·205	—	·164	—	·140	—	·066	—	·220	—	·162	—	·152	—
Calcium oxide (CaO) ...	—	—	—	—	—	—	—	—	—	—	·38	—	—	—	—	—
Magnesium oxide (MgO) ...	—	—	—	—	—	—	—	—	—	—	·19	—	—	—	—	—
Insoluble ...	77·20	—	70·7	—	69·22	—	74·05	—	73·9	—	70·9	—	72·8	—	70·02	—
Soluble in 1 % citric:																
Potash (K$_2$O) ...	·017	—	·028	—	—	—	—	—	—	—	·041	—	—	—	—	—
Phosphoric acid (P$_2$O$_5$) ...	·008	—	·010	—	·010	—	·012	—	—	—	·027	—	—	—	—	—

TABLE IV (continued). *Carnarvonshire Stony Loams.*

	Sub-type B						Sub-type C					
	Douglas Hill C30		Rhyd-ddu C45		Pentyrch C43		Griffith's Crossing C31		Bangor C37		Bangor C38	
	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil
Fine gravel	11·84	7 65	18·03	4·56	12·44	14·71	18·87	46·79	9·03	19·83	16·72	29·53
Coarse sand	14·59	13·66	13·26	11·68	10 04	9·67	21·63	13·58	22·14	17·44	19·85	13·79
Fine sand	23·23	19·10	21·81	33·99	20·03	23·20	22·15	8·98	20·04	18·43	19·07	12·18
Silt	12·42	19·15	11·66	11·47	14·03	18·29	9·74	1·36	10·40	11·49	8·66	9·28
Fine silt	16·69	22·34	19·85	23·37	18·19	12·72	12·74	9·45	14·71	14·39	14·39	14·32
Clay	2·44	3·25	1·64	2·44	5·12	7·16	2·84	1·55	3·44	2·63	3·04	2·79
Moisture	2·88	2·20	1·02	1·62	6·20	4·20	2·30	1·32	3·94	3·34	3·20	3·36
Organic matter	15·32	10·80	13·06	9·78	12·84	9 30	11·92	9·48	15·52	15·62	14·42	13·94
Calcium carbonate	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Nitrogen	·532	—	·448	—	·438	—	·420	—	·641	—	·602	—
48 hours' digestion with HCl:												
Potash (K_2O)	·628	—	·550	—	·599	—	·564	—	·912	—	·928	—
Phosphoric acid (P_2O_5)	·212	—	·230	—	·184	—	·188	—	·298	—	·314	—
Calcium oxide (CaO)	—	—	—	—	—	—	—	—	—	—	—	—
Magnesium oxide (MgO)	—	—	—	—	—	—	—	—	—	—	—	—
Insoluble	73 85	—	61·2	—	69 32	—	75 6	—	70·2	—	65·7	—
Soluble in 1 % citric:												
Potash (K_2O)	—	—	—	—	—	—	—	—	—	—	·017	—
Phosphoric acid (P_2O_5)	—	—	—	—	·013	—	—	—	—	—	·040	—

TABLE IV (continued). *Carnarvonshire Stony Loams.*

| | Sub-type D | | | | | | Unclassified | | | | | |
| | Llanllyfni C15 | | Fron-grasanog C17 | | Pant-glas C18 | | Chwilog C9 | | Chwilog C10 | | Pennant C26 | |
	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil
Fine gravel	11·39	24·72	12·41	24·80	12·20	20·29	6·89	—	10·31	—	3·75	—
Coarse sand	15·00	19·05	10·36	13·83	17·17	16·57	12·58	—	14·52	—	4·83	—
Fine sand	16·36	5·96	13·37	9·06	16·08	9·72	31·39	—	27·77	—	5·16	—
Silt	5·95	19·52	9·63	18·90	8·85	23·85	8·00	—	9·58	—	23·17	—
Fine silt	21·43	8·72	20·60	13·27	21·90	12·24	19·46	—	17·94	—	34·59	—
Clay	5·09	6·71	5·80	4·53	6·06	5·30	5·01	—	3·88	—	3·80	—
Moisture	3·56	1·69	7·20	3·42	4·14	2·24	3·58	—	2·36	—	3·28	—
Organic matter	16·64	6·30	20·34	7·26	11·28	8·20	10·76	—	11·70	—	25·42	—
Calcium carbonate	Nil	Nil	Nil	Nil	Nil	Nil	Nil	—	Nil	—	Nil	—
Nitrogen	·490	—	·600	—	·450	—	·357	—	·385	—	1·08	—
48 hours' digestion with HCl:												
Potash (K_2O)	·251	—	·447	—	·370	—	·550	—	·700	—	·846	—
Phosphoric acid (P_2O_5)	·144	—	·119	—	·175	—	·178	—	·206	—	·186	—
Calcium oxide (CaO)	—	—	—	—	—	—	·22	—	·20	—	—	—
Magnesium oxide (MgO)	—	—	—	—	—	—	·55	—	·35	—	—	—
Insoluble	71·5	—	64·2	—	73·0	—	70·4	—	71·2	—	62·9	—
Soluble in 1 % citric:												
Potash (K_2O)	·026	—	·030	—	·027	—	—	—	—	—	—	—
Phosphoric acid (P_2O_5)	·017	—	·018	—	·015	—	—	—	—	—	—	—

Wind-blown Sands.

These are well developed in North Wales. In South Carnarvonshire such sands are found between Criccieth and Portmadoc and at intervals to the west, notably near Pwllheli and Abersoch. The greatest development of blown sand is seen along the south coast of Anglesey and the Carnarvonshire coast immediately opposite. Small dunes are also found near Deganwy. The great sand areas of Flintshire, not being associated with Palæozoic soils, fall outside the scope of the present paper.

Cultivation on the wind-blown sands is confined to Morfa Bychan between Criccieth and Portmadoc and in the vicinity of Newborough in Anglesey, where small market garden holdings occur. How far this cultivation might be extended it is hard to say, but the following analyses are instructive. A 51 is a soil from a small holding near Newborough where passable crops of potatoes and carrots are grown. It is typical of a small and fairly prosperous group of holdings. A 2 is a sample of soil from the adjacent Newborough warren covered by a thin vegetation of *Salix repens* and other sand plants.

	A 51	A 2
Fine gravel	·00	·00
Coarse sand	93·72	94·33
Fine sand	2·81	2·46
Silt	·49	·29
Fine silt	·38	·32
Clay	·00	·00
Moisture	·38	·54
Organic matter ...	2·38	1·70
Nitrogen	·084	·049
K_2O	·120	·141
P_2O_5	·048	·047
K_2O } soluble in 1 %	·009	·010
P_2O_5 } citric acid ...	·011	·019
Calcium carbonate ...	Nil	·58

It would certainly appear remarkable that a soil containing over 90 % of coarse sand should be able to furnish a livelihood. Two circumstances may be mentioned in explanation. The rainfall is about 35 inches per annum and also there is a permanent water-table within three or four feet of the surface[1].

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[1] Cf. Jean Jonescu, *Agricultura Romănă*, "Les qualités du sol varient d'après le climat: par exemple, un terrain sablonneux et brûlant produit de bonnes récoltes dans la zone montagneuse où il pleut suffisamment, tandis que dans la région des plaines, il ne produit rien."

The average composition of five soils of this class is shown in the general table.

Calcium carbonate only occurred in one sample.

TABLE V. *Wind-blown Sands.*

	Newborough A 2		Penlôn, New-borough A 51		Abersoch C 11		Dinlle C 22		Morfa Bychan C 27	
	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil
Fine gravel	·00	·00	·00	·00	5·75	6·61	·00	·00	·32	·00
Coarse sand	94·33	95·59	93·72	98·05	63·34	66·01	73·86	77·70	75·87	81·94
Fine sand	2·46	1·97	2·81	1·18	9·67	9·75	20·04	19·52	15·30	15·48
Silt	·29	·12	·49	·23	6·69	8·15	1·22	·25	1·05	·35
Fine silt	·32	·18	·38	·43	6·64	3·78	1·07	·97	·80	·55
Clay	·00	·00	·00	·00	1·18	1·61	·00	·00	·00	·00
Moisture	·54	·18	·38	·14	1·46	·82	·52	·36	·40	·04
Organic matter	1·70	·98	2·38	·82	5·52	2·70	2·56	1·60	3·58	·84
Calcium carbonate	·58	1·26	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Nitrogen	·049	—	·084	—	·188	—	·198	—	·162	—
48 hours' digestion with HCl :										
Potash (K_2O)	·141	—	·120	—	·340	—	·169	—	·147	—
Phosphoric acid (P_2O_5) ...	·047	—	·048	—	·122	—	·053	—	·046	—
Calcium oxide (CaO) ...	·47	—	—	—	—	—	—	—	—	—
Magnesium oxide (MgO)...	—	—	—	—	—	—	—	—	—	—
Insoluble	90·5	—	91·2	—	85·5	—	92·4	—	94·2	—
Soluble in 1 % citric :										
Potash (K_2O)	·010	—	·009	—	·027	—	—	—	—	—
Phosphoric acid (P_2O_5) ...	·019	—	·011	—	·006	—	·017	—	—	—

Alluvium.

There are two classes of alluvial soil in North Wales, namely (*a*) estuarine, and (*b*) fluviatile.

(*a*) *Estuarine Alluvium* occurs in portions of estuaries or arms of the sea which have been reclaimed by artificial or natural embankments. The largest of these stretches is Malltraeth marsh in Anglesey which has an area of several thousand acres and runs well into the centre of that island. It was reclaimed by embankment and drainage over a century ago. Unfortunately many of the drains are working very inefficiently and the land is at present waterlogged during the wet season. If the drainage could be made efficient a large area of land would be greatly increased in value. The other large area of reclaimed estuary is at Portmadoc. This was reclaimed by an embankment over a mile

TABLE VI. *Estuarine Alluvium.*

	Malltraeth A19		Ystumllyn C13		Dinas Dinlle C23		Portmadoc C24		Tremadoc C25		Llanfaglan C34	
	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil
Fine gravel	·16	·35	3·93	0·68	·00	·00	·46	1·01	1·20	·73	·53	·47
Coarse sand	8·34	11·38	47·76	67·85	15·58	5·62	18·04	49·50	3·37	2·82	47·52	68·34
Fine sand	40·66	47·13	18·85	12·83	21·19	10·05	69·27	44·12	57·45	65·16	15·48	10·65
Silt	18·09	13·40	5·15	2·65	19·10	22·40	2·36	·51	7·09	5·45	10·05	5·21
Fine silt	13·38	11·71	4·96	2·65	23·43	34·11	1·37	1·07	18·00	16·53	13·61	7·78
Clay	8·41	9·31	1·37	·76	10·28	17·60	·50	·00	2·76	4·88	3·79	3·33
Moisture	3·08	2·32	3·20	·62	2·15	2·68	·66	·25	1·25	·54	1·18	·90
Organic matter	5·64	2·84	12·56	2·52	6·86	5·20	4·02	1·84	7·12	3·60	5·70	3·04
Calcium carbonate	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Nitrogen	·211	—	·496	—	·270	—	·140	—	·200	—	·258	—
48 hours' digestion with HCl·												
Potash (K_2O)	·466	—	·432	—	·801	—	·178	—	·418	—	·536	—
Phosphoric acid (P_2O_5)	·109	—	·143	—	·110	—	·078	—	·142	—	·095	—
Calcium oxide (CaO)	·34	—	—	—	—	—	—	—	—	—	—	—
Magnesium oxide (MgO)	·31	—	—	—	—	—	—	—	—	—	—	—
Insoluble	80·4	—	69·3	—	78·12	—	85·6	—	83·4	—	87·53	—
Soluble in 1 % citric :												
Potash (K_2O)	·017	—	·031	—	—	—	—	—	—	—	·013	—
Phosphoric acid (P_2O_5)	·035	—	·007	—	—	—	—	—	—	—	·019	—

TABLE VII. *Fluviatile Alluvium.*

	Dolben D7		Lleweni D10		Rhewl D26		Llanrwst D51		Maenan C49		Llangerniew D60	
	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil
Fine gravel ...	1·11	2·05	·69	·54	9·09	4·01	1·57	·70	·30	—	13·73	12·44
Coarse sand ...	12·40	13·51	·54	1·99	11·74	12·41	3·04	1·04	·49	—	17·06	21·27
Fine sand ...	20·71	19·54	2·51	2·90	21·34	19·90	11·58	9·96	17·70	—	13·32	12·56
Silt ...	21·96	22·45	9·26	12·69	18·37	31·76	20·80	32·36	18·41	—	24·30	29·60
Fine silt ...	25·89	25·76	57·59	50·82	25·06	18·73	45·60	40·46	38·60	—	16·83	12·02
Clay ...	3·45	5·17	13·90	19·47	4·47	5·07	4·60	4·66	9·54	—	3·66	3·18
Moisture ...	3·62	4·28	2·74	3·10	2·04	1·14	2·10	2·20	2·72	—	1·64	1·08
Organic matter ...	7·62	9·86	9·52	6·94	9·50	4·72	9·22	7·74	9·80	—	8·16	6·00
Calcium carbonate ...	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	—	—	—
Nitrogen ...	·280	—	·302	—	·431	—	·336	—	—	—	—	—
48 hours' digestion with HCl :												
Potash (K₂O) ...	·890	—	·982	—	1·020	—	·720	—	·736	—	—	—
Phosphoric acid (P₂O₅) ...	·115	—	·109	—	·134	—	·190	—	·136	—	—	—
Calcium oxide (CaO) ...	·30	—	·28	—	—	—	—	—	—	—	—	—
Magnesium oxide (MgO)...	·19	—	·58	—	—	—	—	—	—	—	—	—
Insoluble	73·3	—	68·8	—	74·2	—	62·2	—	72·1	—	—	—
Soluble in 1 % citric :												
Potash (K₂O) ...	·031	—	·012	—	·028	—	·028	*	—	—	—	—
Phosphoric acid (P₂O₅) ...	·008	—	·010	—	·008	—	·006	—	—	—	—	—

long nearly a century ago[1]. The town of Portmadoc and the village of Tremadoc are situated on this former estuary—still known as Traeth Mawr. Agriculturally and otherwise the reclamation of Traeth Mawr has not been remunerative. It is possible however that the sandy soils may be suitable for market gardening. At present they carry rather poor grass.

Small areas of this type of alluvium occur also at Ystumllyn near Criccieth where a shingle bank has cut off a creek from the sea leaving about 200 acres of poor sandy soil often flooded in wet weather, and at Llanfaglan near Carnarvon where small areas have been reclaimed around Foryd Bay.

As may be expected, from their origin, the estuarine alluvia show considerable variations in composition. An average analysis will therefore have no meaning. The analyses of two extreme types are shown in the general table. C23 is a fairly heavy loam from Dinas Dinlle near Foryd Bay; C24 is a sandy soil from Portmadoc. Both are under grass. It is noteworthy that the grass on C24, in spite of the coarse nature of the soil, is passable in quantity and quality. The rainfall here is over 45 inches per annum.

(b) *Fluviatile Alluvium* occurs in the valleys of Conway, Clwyd and Elwy. In the first two of these valleys the alluvium occupies a considerable area—probably well over one thousand acres in each case. In the valley of the Elwy the alluvium is confined to thin strips along the river bank. These soils are chiefly under permanent grass and are considered fertile, particularly in the Conway valley. In the valleys of Conway and Clwyd considerable tracts of waste occur where the drainage is imperfect.

In composition they show considerable variation and no purpose will be served by giving the average analyses. Three sub-types can however be recognised and an analysis of a soil of each type is given.

Dolben D7 is typical of the lighter type of alluvium found along the banks of the Elwy and in the upper parts of the Vale of Clwyd. Llanrwst D51 is typical of the Conway alluvium and is distinguished by its high proportion of silt and fine silt. It contains comparatively little clay and is fairly easily worked. Lleweni D10 is typical of a large stretch of heavy alluvium in the Denbigh district. As will be seen, it contains a remarkably high proportion of fine silt and, for North Wales, a large amount of clay. It is, agriculturally, a true clay.

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[1] This embankment forms the subject of several pages of Thomas Love Peacock's *Headlong Hall.*

No calcium carbonate has been found in any of the estuarine or fluviatile alluvia.

Peat Soils.

The North Wales peats fall into two main classes namely shallow peats and deep peats.

I. *Shallow peats* occur over a large part of the uplands of Denbighshire and in western Carnarvonshire. They consist of a thin layer of peat (up to 12 inches) lying on gravelly loam or clay subsoils. A provisional subdivision may be made into (*a*) the Denbighshire type, occurring from 600 feet to 1200 feet lying over Silurian and Ordovician shale subsoils, and (*b*) the Carnarvonshire type occurring from 100 feet to 800 feet and lying over glacial drift subsoils. Both sub-types are little cultivated but in certain favoured spots successful reclamation has been carried out. As might be expected, cultivation leads to a diminution in the content of organic matter. This is well shown by the comparison of two soils from Llanllyfni. C 15 was taken from a root field in a small holding, while C 16 was taken from the unenclosed waste on the other side of the boundary wall.

		C 15		C 16	
Moisture	... ...	3·56	per cent.	7·58	per cent.
Organic matter	...	16·64	,,	43·06	.
Nitrogen	... ...	·490	,,	1·106	.,
K_2O soluble in HCl	{	·426	,,	·420	,,
P_2O_5	{	·144	,,	·136	,
K_2O soluble in 1 %		·026	,,	·024	,,
P_2O_5 citric acid		·017	,,	·010	,,

The average composition of five peats of this class is shown in the general table. It will be seen that the figures for organic matter fall within fairly close limits. The organic matter in the subsoils, as may be expected in shallow peats, is much less than that in the soils.

Calcium carbonate is invariably absent.

II. *Deep peats.* The continental workers on peat distinguish three stages in the building of deep peat. They are, in vertical succession, (*a*) Fen, Flachmoor, or "Niedermoor," (*b*) a transition type known as Übergangsmoor, and (*c*) Hochmoor. All three types can be observed in North Wales. A thorough investigation of the deep peats will more appropriately form the subject of another paper and, for the present, outlines only can be given.

(*a*) *Fen.* Only one true fen peat has been examined in the laboratory. It was of the typical "Niedermoor" structure and composition,

containing 3·72 % CaO and 1·37 % calcium carbonate. Other peats have however beèn recognised in the field which belong genetically to this type but, since they are fed by water from strata containing very little soluble matter, they are different in vegetation and composition from true fens and approximate more nearly to the Hochmoor type[1].

TABLE VIII. *Shallow Peat.*

	Llanfihangel D 21		Hiraethog D 31		Glan y-Gors C 16		Llangerniew D 62		Rhya-aau C 46	
	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil
Fine gravel ...	—	—	—	—	—	—	—	—	—	—
Coarse sand ...	—	—	—	—	—	—	—	—	—	—
Fine sand ..	—	—	—	—	—	—	—	—	—	—
Silt ...	—	—	—	—.	—	—	—	—	—	—
Fine silt ...	—	—	—	—	—	—	—	—	—	—
Clay ...	—	—	—	—	—	—	—	—	—	—
Moisture ...	7·62	3·34	12·24	—	7·58	—	8·22	8·46	3·04	2·12
Organic matter ...	47·66	17·76	44·78	—	43·06	—	43·44	30·94	41·68	19·28
Calcium carbonate ...	Nil	Nil	Nil	—	Nil	—	Nil	Nil	Nil	Nil
Nitrogen ...	1·23	—	1·39	—	1·11	—	1·18	—	1·34	—
48 hours' digestion with HCl:										
Potash (K_2O) ...	·310	—	·600	—	4·20	—	—	—	·552	
Phosphoric acid (P_2O_5) ...	·153	—	·290	—	·136	—	—	—	·220	
Calcium oxide (CaO)	—	—.	—	—	—	—	—	—	—	—
Magnesium oxide (MgO)...	—	—	—	—	—	—	—	—	—	—
Insoluble ...	40·0	—	35·2	—	45·3	—	—	—	47·64	—
Soluble in 1 % citric										
Potash (K_2O) ...	—	—	—	—	—	—	—	—	—	—
Phosphoric acid (P_2O_5) ...	—	—	—	—	—	—	—	—.	—	—

(b) *Transition moor or Übergangsmoor.* This is not a common type in North Wales and has not been investigated. There are however certain localities where alder and willow can be seen growing on peat and where the development of the peat appears to be tending towards a suppression of these trees.

(c) *Moor*[2] or "*Hochmoor*" is well developed in various places in North Wales. In some peat areas in valleys the development appears to have followed the course Niedermoor[3]—Übergangsmoor—Hochmoor. In other places the peat appears to have developed on wet mountain slopes. Such peats may correspond to the continental Hangmoore[4].

[1] See E. Gully, *Mitt. d. k. Bay. Moorkulturanstalt,* vol. III, 1909, pp. 1–38.

[2] A. G. Tansley, *Types of British Vegetation,* p. 211; W. Bersch, *Handbuch der Moorkultur,* p. 11 et seq.

[3] Niedermoor is not used in Weber's sense as corresponding to Fen, since calcium carbonate is generally lacking from telluric waters in North Wales. Flachmoor would probably be more exact. (See Tansley, *loc cit.*)

[4] Ramann, *Bodenkunde,* p. 186.

They are never found in cultivation and at best carry an inferior grass herbage.

The average composition of three samples of Deep peat is given.

The deep peats are notably deficient in potash. It will be seen that the subsoil is slightly richer in organic matter than the soil. This is not only true for the average but for the individual samples.

TABLE IX. *Deep Peat.*

	Cors-y-bol A 46		Golan C 28		Nant Ffrancon C 47		Bodeilio A 17		Maenan C 50	
	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil
Fine gravel	—	—	—	—	—	—	—	—	—	—
Coarse sand	—	—	—.	—	—	—	—	—	—	—
Fine sand	—	— -	—	—	—	—	—	—	—	—
Silt	—	—	—	—	—	—	—.	—	—	—
Fine silt	—	— -	—	—	—	—	—	—	—	—
Clay	—	—	—.	—	—	—	—	—	—	—
Moisture	8.32	11.94	7.68	12 38	7.00	12.76	16.32	12.40	9.48	—
Organic matter	75.28	79.52	68.88	79.00	71.18	79.94	54.82	35.40	52.16	—
Calcium carbonate	Nil	Nil	Nil	Nil	Nil	Nil	1.37	1.64	Nil	—
Nitrogen	2.23	—	1.58	—	1.94	—	1.45	—	1.50	—

48 hours' digestion with HCl.

Potash (K$_2$O)	.230	—	.217	—	.268	—	.277	—	.400	—
Phosphoric acid (P$_2$O$_5$) ...	.154	—	.147	—	.149	—	.133	—	.155	—
Calcium oxide (CaO) ...	.80	—	—	—	—	—	3.72	—	—	—
Magnesium oxide (MgO)...	.25	—	—	—	—	—	.13	—	—	—
Insoluble	14.4	—	14.6	—	14.08	—	17.8	—	33.60	—
							Fen Peat		Fringing Alluvium	

Soluble in 1 % citric :

Potash (K$_2$O)	—	—	—	—	—	—	—	—	—	—
Phosphoric acid (P$_2$O$_5$) ...	— -	—	—	—	—	—	—	—	—	—

CONCLUSIONS.

The main types of Palæozoic soils in North Wales having now been described it remains to consider in what respects they resemble each other and to note in what respects they differ from other soils studied hitherto.

1. In mechanical composition it will be noticed that with the exception of sands, alluvia and peats, the soils of this area are of a loam texture. Sandy soils are only found near the sea coast and clay soils are very rare. With the high rainfall, however, many of the heavier loams present to the cultivator the difficulties of clay soils. Looking

over the analyses it will be seen that the clay fraction rarely exceeds 10 % in the case of soils derived from the shale or 7 % in the case of the Anglesey and Carnarvonshire loams. Usually the clay fraction falls considerably below these figures. The silt fractions, on the other hand, particularly in the Palæozoic silt loam, form a considerable proportion of the soil.

These circumstances, together with the high proportion of organic matter, render the North Welsh soils easy to work where water conditions are not unfavourable. A number of determinations were made of the plasticity-numbers of various typical soils according to the method of Atterberg[1]. The plasticity-numbers for shale soils varied from nothing to 10. In the case of the Anglesey medium loams no appreciable plasticity was found. The heaviest of the Carnarvonshire stony loams gave a value 7·2. According to Atterberg's standards, these soils cannot be reckoned heavier than medium loams.

The high proportions of silt and fine silt render soils sticky when wet, but on drying they are generally quite friable. In order to determine the effect of fine silt on the plastic properties of a soil a glacial clay from the vale of Clwyd, containing 34·4 % fine silt and 14·9 % clay, was taken and the clay removed by sedimentation. The residue was dried and the following plasticity numbers were obtained by the Atterberg method:

D 19 B	D 19 B (clay removed)	D 19 B (clay fraction)
23·9	0	31·3

It is of course probable that the high proportions of organic matter contribute to render soils friable on drying.

With regard to the plasticity of soils and the factors producing it, two main theories may be noted. On the one hand there is the theory that plasticity is due to the presence in soils of hydrated colloidal products. This theory has been recently discussed by P. Rohland[2]. If this theory be correct the general absence of plasticity in the Palæozoic soils may be explained. The soils examined in this paper are derived from three main types of rocks, namely, the igneous and volcanic rocks of Carnarvonshire, the metamorphic rocks of Anglesey and the sedimentary rocks found all over the area. From the nature of the first two rock types they cannot be expected to contain pre-formed colloid materials and whatever colloid clay material is found in the soils derived

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[1] A. Atterberg, *Int. Mitt. für Bodenkunde*, vol. I, p. 10.

[2] P. Rohland, *Die Tone*, Vienna, 1910.

from them must be of comparatively recent origin.   The sedimentary rocks present a different problem.   The shales of Cambrian, Ordovician and Silurian age are among the oldest sediments of the Geological record. While they are not metamorphic in the same sense that the Anglesey rocks are metamorphic it must be recognised that they differ considerably from the corresponding sediments of, say, Cretaceous age.   Van Hise[1], who is disposed to denote all changes in the constitution of a rock as metamorphic, distinguishes various zones of metamorphism.   Immediately below the permanent water-table is the zone of cementation. Lower still is the zone of regional metamorphism (anamorphism). Considering the enormous thickness of the Palæozoic sediments of North Wales, it will be seen that regional metamorphism must have played an important part in determining the present structure and composition of these rocks, so that although they may originally have consisted of mud and clay, their subsequent treatment has largely led to the decomposition of whatever colloid materials were present.

Another theory as to soil plasticity is that represented by Atterberg[2].   This theory supposes that plasticity is due to the presence of minute flake-like (schüppenformig) particles.   Atterberg mentions certain minerals which can, when in a fine state of division, display plasticity.   Among these are kaolinite, talc, serpentine, biotite, muscovite, limonite and haematite.   The relation of the mineralogical composition of the North Wales rocks to the plasticity of their resultant soils can only be settled by a minute investigation.   Until such an investigation has been made it is scarcely safe to decide on Atterberg's theory or its application to the area in question.

2.   Sedentary soils and soils derived directly from local drift deposits contain remarkably high proportions of fine gravel.   This is particularly the case in the subsoils.   One sedentary soil in Carnarvonshire contained over 40 % of fine gravel in the subsoil.   In the soils hitherto examined in other parts of Britain the fine gravel is usually by far the smallest fraction except in the case of sands which contain very little clay.

3.   Microscopic examination of the fractions obtained in mechanical analysis shows that the coarser fractions are mainly formed of undecomposed parent rock.   The sand fractions are quite different in

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[1] Van Hise, *Treatise on Metamorphism*, U.S. Geol. Survey Monograph, XLVII.

[2] A. Atterberg, *Int. Mitt. für Bodenkunde*, vol. III, p. 1.

character from the sand fractions obtained from English soils which consist mainly of quartz granules.

In order to get further light on the structure and composition of North Welsh Palæozoic soils, a number of analyses were made of the fractions obtained in mechanical analyses. Determinations were made in each case of Silica (SiO_2), Ferric oxide (Fe_2O_3) and Alumina (Al_2O_3). Soils from each of the first three types described were thus examined and the results are shown in Table X.

TABLE X. *Percentage composition of soil fractions.*

Percentage of SiO_2 etc. in fractions

Soil type	Soil	Constituents	Fine gravel	Coarse sand	Fine sand	Silt	Fine silt	Clay
Anglesey medium loam	A 5	SiO_2	78·6	84·7	81·6	73·8	59·4	45·3
		Al_2O_3	11·2	5·8	7·0	14·8	23·1	35·0
		Fe_2O_3	8·4	5·2	5·0	7·0	11·5	13·2
	A 18	SiO_2	78·7	79·7	83·7	80·4	64·5	48·7
		Al_2O_3	12·4	5·3	4·4	11·6	14·2	30·2
		Fe_2O_3	4·0	5·2	4·2	5·3	10·6	11·2
	A 56	SiO_2	71·3	87·0	82·1	72·2	54·4	46·0
		Al_2O_3	11·4	5·4	6·5	18·8	22·3	30·8
		Fe_2O_3	5·8	4·0	3·7	7·6	12·3	15·3
Palæozoic silt loam	D 18	SiO_2	63·0	68·0	75·1	78·3	66·3	48·1
		Al_2O_3	19·4	14·4	12·9	17·0	22·5	30·5
		Fe_2O_3	10·2	10·2	7·9	5·0	8·5	12·9
	D 33	SiO_2	64·7	75·2	78·2	73·8	69·4	42·4
		Al_2O_3	17·5	14·4	15·2	16·4	14·9	30·2
		Fe_2O_3	10·9	8·0	5·8	8·6	8·3	16·0
	D 45	SiO_2	63·9	58·8	74·3	77·2	67·5	49·5
		Al_2O_3	18·6	20·5	12·8	16·9	18·6	31·9
		Fe_2O_3	16·8	9·0	8·8	5·5	9·8	12·3
	D 55	SiO_2	66·8	68·2	72·0	79·5	65·7	47·6
		Al_2O_3	11·4	11·2	13·4	13·5	19·6	33·2
		Fe_2O_3	8·6	7·4	6·8	5·5	10·0	14·0
	C 14	SiO_2	64·6	67·0	75·8	74·7	62·9	43·8
		Al_2O_3	13·9	16·8	12·5	14·9	21·8	36·4
		Fe_2O_3	10·5	8·4	6·9	7·5	11·5	13·2
Carnarvonshire stony loam	C 18	SiO_2	71·9	83·2	79·1	74·9	66·8	43·0
		Al_2O_3	16·7	7·2	11·4	18·8	21·2	41·4
		Fe_2O_3	5·9	4·2	4·6	5·6	7·0	11·0
	C 31	SiO_2	78·0	82·1	80·5	78·4	65·8	48·0
		Al_2O_3	13·6	6·6	14·5	11·8	13·4	30·2
		Fe_2O_3	4·5	3·8	4·4	7·2	12·0	12·7

The results present several points of interest. Hendrick and Ogg[1] have recently studied the fractions of a Scottish drift soil consisting mainly of local granite and metamorphic material. The soils examined by the writer present many points of similarity to the Craibstone soil and may be contrasted with the English soils submitted to fractional analysis by Hall and Russell[2].

For the sake of comparison the results for the Craibstone and English soils respectively are reproduced in the following table:

Percentages of

	SiO_2		Al_2O_3		Fe_2O_3	
	Craibstone	English	Craibstone	English	Craibstone	English
Fine gravel	84·96	94·4	8·56	3·0	1·10	2·1
Coarse sand	83·92	93·9	9·34	1·6	1·12	1·2
Fine sand	73·87	94·0	13·47	2·0	4·21	1·2
Silt ...	70·15	89·4	14·04	5·1	5·86	1·5
Fine silt ...	67·21	{ 84·1 { 64·3	18·91	{ 7·2 { 19·3	7·85	{ 2·6 { 7·6
Clay ...	44·08	{ 53·2 { 49·0	27·64	{ 21·2 { 29·8	21·81	{ 13·2 { 13·1

The soils examined by the writer agree with the Craibstone soil in being poorer in silica and richer in alumina and ferric oxide in the various fractions. In the Welsh soils however the silica percentages in the coarser fractions are even lower than the silica percentages in the Craibstone soils. The proportion of alumina and ferric oxide are also higher. The most notable difference however is that in the Welsh soils the most siliceous fraction is never the fine gravel as in the Craibstone and English soils. It will be seen that the highest percentage of silica is found in the coarse sand in four cases, in the fine sand in three cases and in the silt three cases.

If we take the average silica percentage in the fine gravel of those soils in which the highest silica percentage is found in the coarse sand, we shall find it to be greater than the corresponding percentage for those soils in which the fine sand is the most siliceous fraction. And similarly the soils in which the fine sand is the most siliceous fraction have a more siliceous fine gravel than the soils in which the highest silica content is in the silt. The following table will illustrate this:

Most siliceous fraction	Average composition of fine gravel		
	SiO_2	Al_2O_3	Fe_2O_3
Coarse sand	75·0	13·2	6·15
Fine sand	69·3	14·6	8·5
Silt ...	64·6	16·5	11·9

Now on microscopical examination it is seen that the fine gravel and, to a large extent, the coarse sand, consist of unaltered rock. In one case, D 18, a sample of the parent rock was obtained and found on analysis to have precisely the same composition (estimating SiO_2, Al_2O_3 and Fe_2O_3) as the fine gravel of the derived soil. Had other samples of parent rock been available this would doubtless have been the case in all the samples, for the fine gravel is usually composed of angular rock fragments. More experimental work is needed but the analyses of the ten sets of soil fractions do suggest that for North Wales at any rate, the more siliceous the parent rock the larger are the particles of highest silica content; and, on the other hand, the lower the content of silica in the parent rock, the finer are the particles containing the highest proportions of silica. It is proposed to enquire into this more closely, performing complete analyses of the fractions.

Considering that the North Welsh Palæozoic soils contain such a large amount of undecomposed rock material even in the material of smaller grades than the fine gravel, it would appear that they form a class similar to that described by Ramann as Steinboden, i.e. soils which consist mainly of slightly decomposed rock fragments[1]. Ramann further divides these soils into three sub-classes according to the size of the rock particles. In North Wales, all gradations can be found from masses of bare boulders to soils in which very little coarse material is found. Ramann believes soils of this class to be the result of mechanical weathering and it would appear that this is true to a large extent for the North Welsh soils. It should be added that Ramann's Steinböden are soils from mountainous areas.

It is worthy of notice that the clay of North Welsh soils shown in the Table contain generally smaller percentages of silica and higher percentages of alumina and ferric oxide than the most extreme types quoted by Hall and Russell[2] for infertile soils. It should be stated that, of the soils whose fractions were examined, all except D 18, D 33, D 45 and C 18 were of soils of considerable fertility.

4. The North Welsh soils are generally deficient in calcium carbonate. It is very rarely that one meets with this soil constituent except in limestone soils. This poverty in calcium carbonate is readily understood when it is considered that limestone scarcely occurs in the rocks of North Wales of Silurian and earlier periods. A few outcrops of limestone are found associated with the Pre-Cambrian rocks of Anglesey.

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[1] Ramann, *Bodenkunde*, 1911, p. 543. Steinboden sind Bodenarten, die sich überwiegend aus wenig zersetzten Gesteinsbruchstücken zusammensetzen.

[2] This *Journal*, vol. IV, pp. 181-223.

Calcareous volcanic ashes occasionally occur in the mountain area, while in the sedimentary rocks only occasional thin bands of limestone are found in the Silurian strata. Where soils have been given large dress- ings of lime, as was formerly the case in Denbighshire, the loss of calcium carbonate in the drainage accounts for its almost complete absence. At Rothamsted about half of the rainfall finds its way into the drainage; in North Wales the proportion must be considerably greater on account of the humidity of the atmosphere and the greater coolness of the summers checking evaporation. With a rainfall of 40 inches per annum in Wales probably 25 inches at least goes into the drainage. The leaching out of soluble constituents must therefore be much greater than it is in the drier districts of England and calcium carbonate may be expected to disappear rapidly from soils under such high rainfalls.

It is remarkable that nitrates can be detected in most of the North Wales soils in spite of the absence of calcium carbonate[1]. In any case it would appear that a type of farming has been developed which dis- penses with lime without any large apparent disadvantages.

Determinations have been made of the lime requirements of a large number of soils by the method of Hutchinson and McLennan. In most cases the deficiency is greater than ·2 % calcium carbonate.

5. The high figures for organic matter are readily explained. On the one hand the humid climate with its mild winters favours vege- tative growth. On the other hand the aerobic decomposition of organic matter in the soil is hindered by the absence of calcium carbonate and the fact that during a large part of the year soils are very poorly aerated consequent on the interstitial spaces of the soil being occupied by water. These circumstances favour anaerobic changes, by which a "sour" peaty humus is produced.

6. The amount of potash in North Wales soils is generally rather high when it is considered that clay soils are so rare. This is not how- ever surprising, since it has been shown that the coarser fractions contain so much unaltered rock and comparatively little quartz. It is not certain whether these soils are benefited by potash manuring. The average of a number of trials made in various parts of the area for a number of years shows comparatively little return for potash manuring.

7. Phosphoric oxide in North Wales soils presents few points of

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[1] The writer is indebted to Mr E. J. Roberts, research student of this college, for observations on this point.

interest. It is fairly high in the Palæozoic silt loams and is not low in any type of soil except the wind-blown sands.

8. From the results obtained, it will be evident that the Palæozoic soils of North Wales differ, not only genetically, but also in their properties and constitution from the soils hitherto studied in this country. Further, the agricultural treatment of them, involving as it does comparatively long periods under grass alternating with arable cultivation, introduces a number of factors which, together with the climate, must be considered in devising schemes for manuring and soil treatment. A considerable amount of experimental work in field and laboratory would therefore appear to be necessary in order to discover to what extent the results of English experiments can be applied to the treatment of North Welsh soils.

THE INFLUENCE OF SOIL CONDITIONS ON THE DECOMPOSITION OF ORGANIC MATTER IN THE SOIL.

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(*Rothamsted Experimental Station.*)

THE biochemical decomposition of plant residues and other organic matter in the soil is of fundamental importance for soil fertility. It causes the breaking down of coarse plant fragments which otherwise might open up the soil too much: it leads to the production of colloidal complexes known as humus which exert many beneficial effects both chemical and physical, and it brings about the formation of nitrates, the most important of the nitrogenous plant nutrients.

The decomposition has been studied extensively in the laboratory and its general course has been fairly satisfactorily determined; but there has not been much work in the field. The difficulties of field work are of course very considerable, but it has the enormous advantage, which in the nature of things laboratory work cannot possess, that it is affected by all the factors concerned, and can therefore be made to reveal them. The detailed study of the way in which each factor operates can then be made in the laboratory.

In large part the decomposition is brought about by bacteria and other micro-organisms. No definite connection, however, has been traced in the field between the activity of the micro-organisms and the extent of the change. It is difficult to measure bacterial activity without using some decomposition and therefore begging the whole question as to its relationship with decompositions: the only method that does not involve this is to count the bacteria by some dilution or plating out method. This has been done by several investigators, but no particular relationship has been observed. In most cases a comparison has been made between different plots of known productiveness with the view

of determining whether the numbers of bacteria on the several plots at a given date corresponded with the order of productiveness. As a rule no correspondence has been observed. Kellermann and Allen's results[1] are as follows:

	Very pro- ductive	Productive			Poor		
Plot No.	—	40	190	290	10	30	180
Bacterial numbers, mil- lions per gram ...	0 02	0·21	0·003	0 27	0·44	0·16	0·06

Percy E. Brown[2] also failed to obtain any close connection:

Plot No.	607	604	602	901	601	609
Yield of maize, bushels per acre	52·7	50·7	46·0	43·2	35·5	32·5
Bacterial numbers, millions per gram ... .:. .	2·8	3·3	2·6	2·5	2·1	2 7

It could hardly be expected, however, that any very close connection would be traced. The productiveness of a plot of land is determined by a number of factors, of which bacterial activity is only one, and, at any given moment, not necessarily a particularly important one.

A better method for studying the connection between bacterial numbers and the extent of the decomposition is to follow the changes on a single plot systematically during a long period, and see how closely the rate of decomposition is related to the changes in bacterial numbers.

This we have done for three seasons, and we have brought the results of our investigations together in the present paper.

We have followed in detail the changes in amount of nitrate in the soil, of CO_2 in the soil air, and of bacterial numbers in the soil. The determinations have been made at frequent intervals, usually some 10 to 14 days, and still more frequently at critical periods, -and the results have then been plotted.

The analytical results do not tell us precisely how much CO_2 and nitrate are formed in the field because CO_2 diffuses out and nitrate washes away to an unknown extent. Any attempt to enclose the soil involves separating it from the main body and putting it under more or less artificial conditions, which would defeat the whole object of the work. For our purpose we mainly want the fluctuations rather than the absolute quantities, and these are shown to a considerable extent by the curves. When the curves are rising production is obviously

[1] *U S Dept. of Agric Bureau of Plant Industry*, Bull No 211, 1911.
[2] *Journ. Research Bull.* **2**, 1911; *Centr. Bakt. Par* 1912, Abt. II, **35**, 234 272; and *Journ. Agric. Research*, 1916, **5**, 855-869.

dominating the situation, when they are falling loss is the dominating factor.

The curves for bacterial numbers, nitrate content, and CO_2 in soil· air, are sufficiently similar to justify the view that all the phenomena are related. Thus, with some exceptions during periods of active plant growth, a rise in bacterial numbers is accompanied by a rise in CO_2 in the soil air, and somewhat later by a rise in nitrate in the soil. Conversely a fall in bacterial numbers is accompanied by a drop in CO_2 evolution and in nitrate accumulation. This occurs so frequently that it cannot be accidental, and we are forced to conclude that the production of CO_2 and of nitrate in the soil is definitely connected with the rises and falls in bacterial numbers. The curves thus afford a demonstration of the fundamental proposition in soil bacteriology, that the rate of decomposition of organic matter in the soil is a function of the bacterial activity.

The curves have further enabled us to ascertain the more important factors at work in determining the amount of change in the soil in natural conditions, and to form some estimate of their relative importance   In order to do this we have made systematic determinations of the soil moisture and soil temperature, and these, as well as the other meteorological data, have been plotted. In so far as the curves for nitrate, CO_2 and bacterial numbers agree with the curve of any one of these factors over a sufficient period to eliminate accidental coincidences, that factor is taken as dominating the situation. When no agreement exists, we suppose that some other factor is operating for which therefore search is made.

The field observations thus show the dominating factors under given conditions, or, alternatively, they show that the dominating factor is not yet discovered, and that further investigation is necessary. They do not, however, indicate very distinctly the nature of the relationship between the various factors and the biochemical reactions. This requires a separate set of investigations to be made in the laboratory under rigidly controlled conditions where all the factors except the one under investigation are kept constant.

The application of this method has led to the following conclusions:

Temperature is the chief factor in determining the extent of the biochemical changes in the soil. Until the temperature exceeds $5°$ C., change is only very slow; from November to March the reactions we are considering seem to be almost at a standstill.

As soon as the temperature begins to rise above $5°$ C. biochemical

activity sets in; bacterial numbers, CO_2, and nitrates all increase, the curves agree so closely with those for temperature that we are justified in regarding the temperature rise as the determining factor. The increased activity is not always equally sustained, nor does it always quite coincide with the rise in temperature; occasionally it follows later.

But this agreement soon ceases, and after a short period the activity begins to fall off notwithstanding the continuance of the favourable temperature. This is not a result of the sustained higher temperature because it is not obtained in laboratory experiments where soils are kept at different constant temperatures, all other conditions being alike[1]. The result seems to be due to lack of moisture, because the curves now begin to resemble the moisture curves. But the moisture curve does not fit very well, and, therefore, it is not the only factor concerned. The rainfall curve fits better. We conclude then, that under favourable temperature conditions, rainfall becomes the dominating factor, but that rainfall does something else besides supplying water, and search was therefore made for this new factor.

The beneficial effect of showers of rain has long been known, and agriculturists have always felt that something more than a moisture effect is concerned. Liebig considered that ammonia was present in sufficient quantities to affect the plant growth, but the many analyses which have been made dispose of this view. As we pointed out in our previous paper[2] our observations suggest that the dissolved oxygen of the rain is the important factor, renewing the oxygen in the dissolved atmosphere of the soil, and thus giving the organisms a new lease of activity. This view is borne out by the circumstance that the effect is most evident on dunged soils and on cropped soils where considerable oxygen is used up; on the poor unmanured soil the effect does not show; indeed it is rather reversed at some periods.

The three factors, temperature, moisture, and the third, which we believe to be the supply of dissolved oxygen, fit the nitrate and CO_2 curves tolerably well over a large part of the year, and thus suffice to account for most of the phenomena. But there still remain two periods which are not thus fitted, and where, therefore, some other factor must be operating; a period of depression coming after the spring rise, and a period of autumn activity coming after the summer sluggishness. We must therefore see what further factors may be involved.

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[1] See E. J. Russell and H. B. Hutchinson, this *Journal*, 1912, **5**, 157 *et seq.*

[2] This *Journal*, 1915, **7**, 24, and this vol. p 331. E. H. Richards in these laboratories has since shown that rain is nearly a saturated solution of oxygen.

By comparing the curves for fallow and for cropped land it is possible to ascertain the effect of the growing crop. So far as the accumulation of nitrate is concerned the effect is markedly depressing. Part of this, however, is due to the nitrate taken by the crop, but after allowance is made for this there still appears to be a depression. The curves do not show how this is caused, whether it is due to the competition of the plant roots for moisture, or for dissolved oxygen, or whether some definite toxin is concerned. The effect is counteracted for a time by a shower of rain, but it soon sets in again.

We therefore regard the growing crop as a fourth factor and on the whole a detrimental factor in the biochemical relationships.

Probably also the amount of CO_2 present in the soil air is another factor, though here the evidence is less clear because the effect of CO_2 only appears when large amounts are present, and this only happens on cropped ground.

But these also do not account for the two periods already mentioned, nor do any other of the factors we have studied.

So far we have dealt with biochemical activity as a whole. We must now discuss the problem a little more closely so as to deal with the separate quantities concerned; the bacterial numbers, the CO_2 content of soil air and the nitrate content of the soil.

It is generally recognised that the biochemical decompositions in the soil are mainly brought about by bacteria, and this view is supported by the fact that a distinct connection can be traced between the bacterial numbers and the production of CO_2. We observed this in our previous paper; this year's results emphasise it. On the Broadbalk dunged fallow, for instance (Fig. 2), where there is no complication arising from the presence of a crop, the curves both for bacterial numbers and for CO_2 rise and then fall in May, rise in July, fall in August and September, rise and fall in October, and then rise in November. There is, however, a discrepancy in June when the numbers fall while the CO_2 rises. Great Harpenden Field (Fig. 5) shows a similar kind of agreement in late March and April, and also from October onwards. The agreement continues so long as the CO_2 is only small in amount, but it breaks down when the CO_2 becomes high as on the cropped ground between May and August, i.e. during the active growing period; the numbers are then depressed and show no rise corresponding with the CO_2 peak.

As they stand the curves for bacterial numbers are not clearly related to those for nitrate. Closer examination shows a similarity but some displacement, the rise in nitrate coming after the rise in bacterial
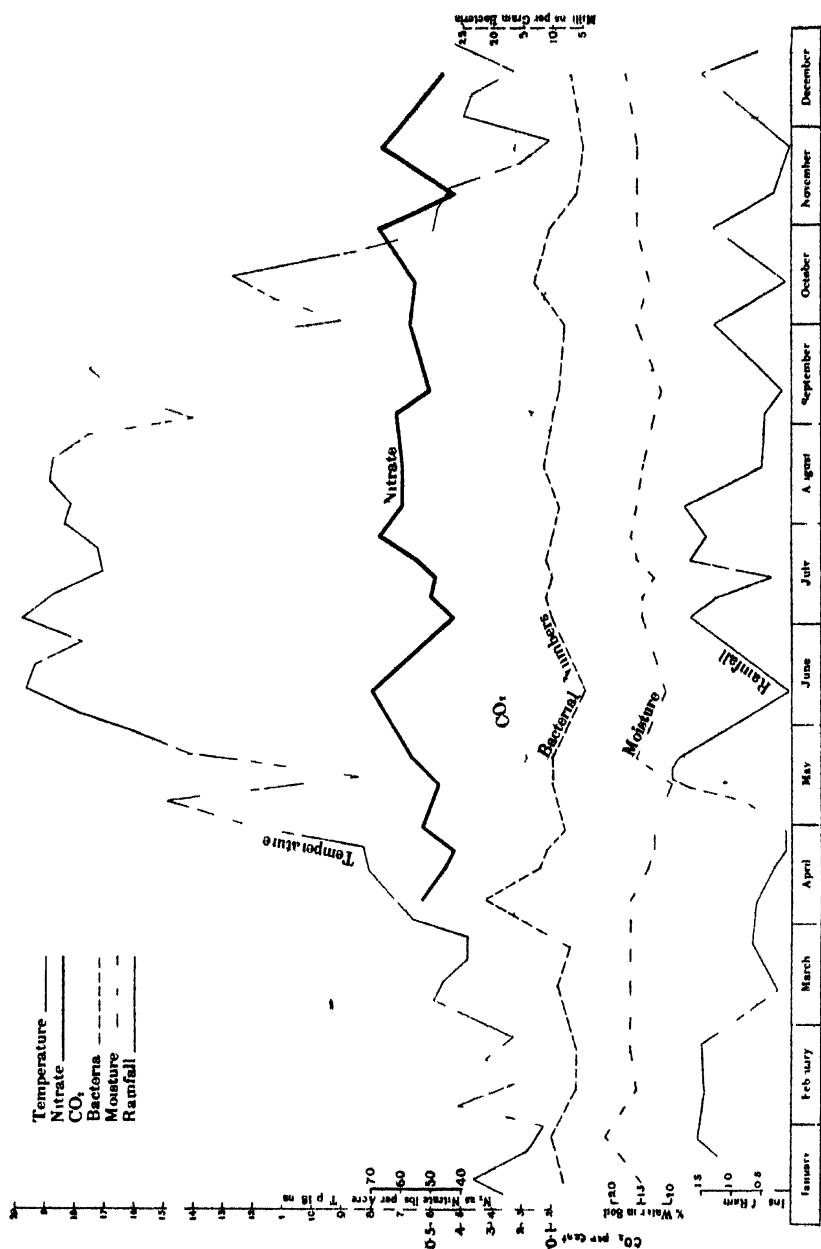
Fig 1   Data for Broadbalk unmanured fallow plot   The temperature is taken at a depth of 6″   The scale on the left rising to 20 gives the temperatures in °C.   The rainfall is for the seven days preceding the determinations
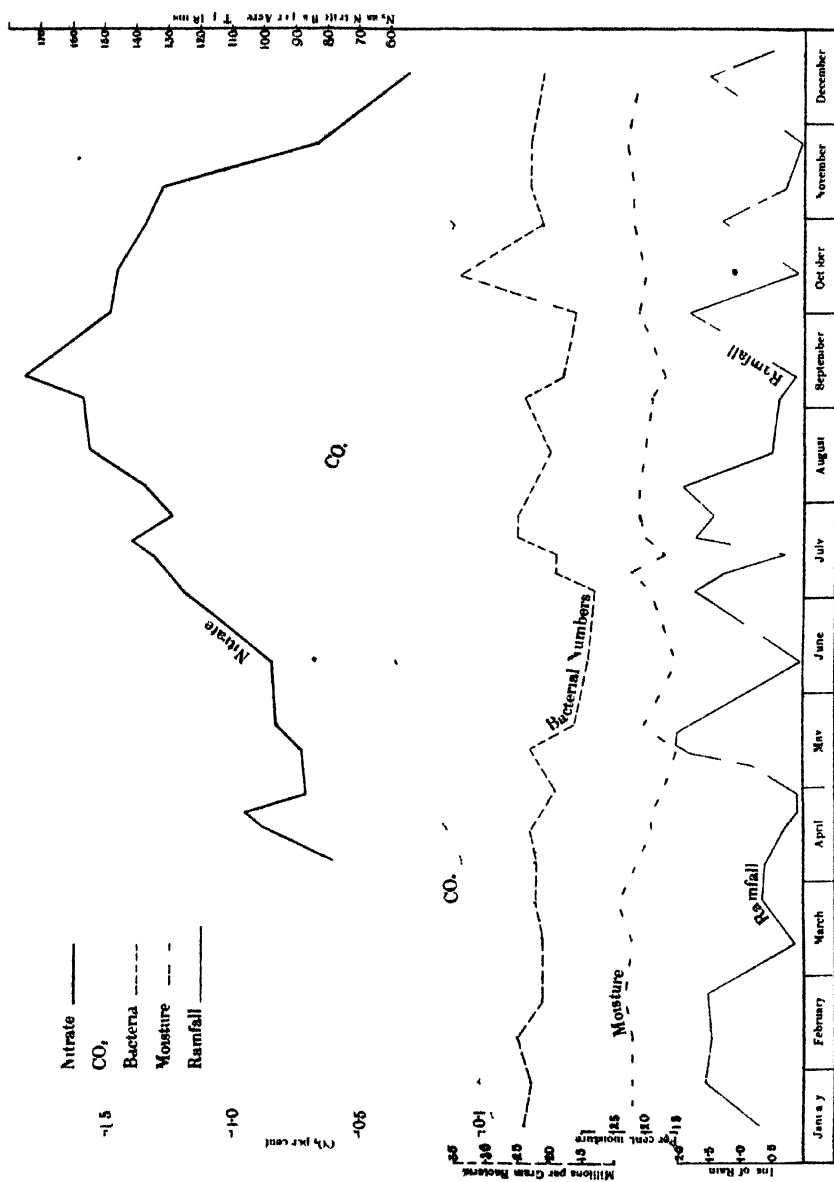
Fig 2   Data for Broadbalk dunged fallow plot
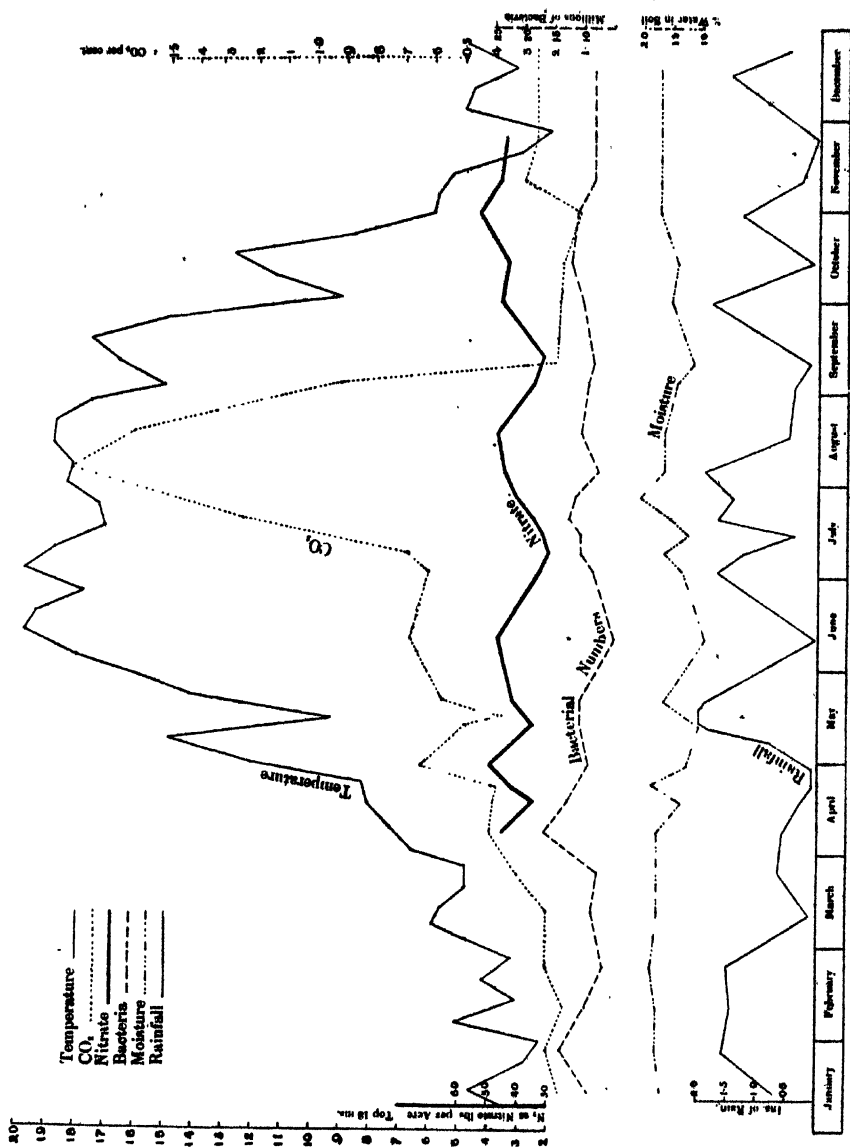
Fig. 3. Data for Broadbalk unmanured cropped plot. The temperature is taken at a depth of 6″. The scale on the left rising to 20 gives the temperature in °C. The rainfall is for the seven days preceding the determinations.
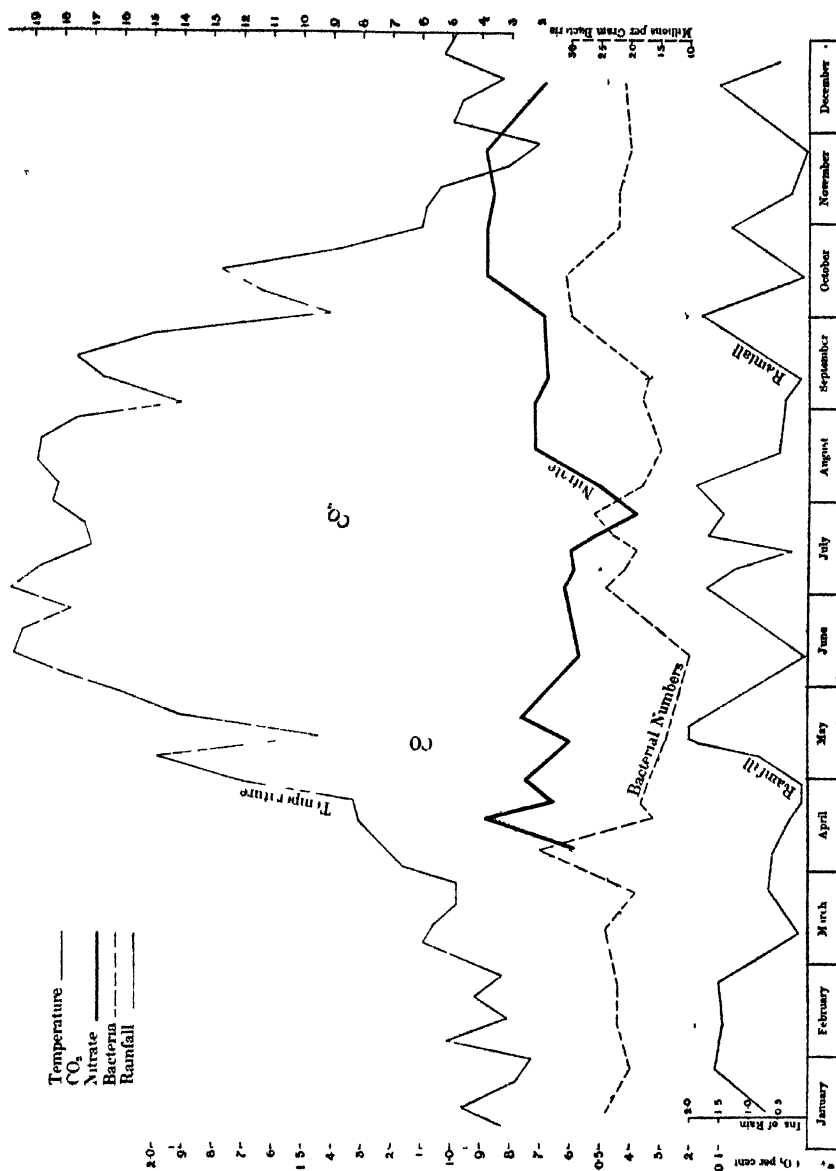
Fig. 4.   Data for Broadbalk dunged cropped plot. The temperature scale is given on the right rising to 19 : the readings are taken at a depth of 6″. The rainfall is for the seven days preceding the determinations.

Fig. 5. Data for Great Harpenden field. The rainfall is for the seven days preceding the determinations.

numbers, and continuing after the numbers have begun to fall. It might be argued that the similarity is only accidental and that the curves for nitrate and for bacterial numbers have nothing to do with one another; in other words that the bacterial numbers as recorded by this method are not related to the decompositions in the soil. This view is, we think, ruled out by the regularity with which the result has been obtained during the last three years (1914—1916). The method of counting is admittedly faulty, and it has not yet reached the precise quantitative stage; before it does this not only must it be improved very considerably, but the part played in the decomposition by moulds and fungi of the soil must be ascertained. In spite of its defects, however, experience shows that it indicates the fluctuations in numbers and gives results in fair agreement with those obtained by other methods[1]. On the whole, therefore, we prefer to explain the phenomena on the assumption that bacteria are the active agents and that the curves are connected. Admitting the truth of the relationship its obvious meaning is that the formation of nitrate is dependent on some previous change, which in turn is dependent on the bacterial numbers.

There are two possibilities; one might suppose that the first change is ammonia production, which goes on simultaneously with the increase in numbers, and that this is followed by nitrate production, which is independent of these particular organisms. The lag between the curve for bacterial numbers and for nitrate would then represent the time required for the conversion of ammonia to nitrate. This view has in its favour the fact that the ammonia-producing bacteria are countable by the gelatine plate method, while the nitrifying organisms are not. But we consider it to be ruled out because it is inconsistent with another fact, already demonstrated here, that the amount of ammonia in the soil is always a minimum, and consequently the rate of conversion of ammonia to nitrate is greater than that of ammonia formation, so that there cannot be a lag. The dividing up of the reaction must therefore go further back and the formation of ammonia supposed to involve two stages, the first being brought about by bacteria capable of growing on the plates, and therefore fluctuating according to the numbers there recorded, while the second is subsequently, and somewhat more slowly, brought about by other organisms or in another way.

This hypothesis cannot be tested in the field, but only in the laboratory; we hope to make the investigation at an early opportunity.

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[1] See E. J. Russell and H. B. Hutchinson, this *Journal*, 1913, 5, 215 *et seq.*
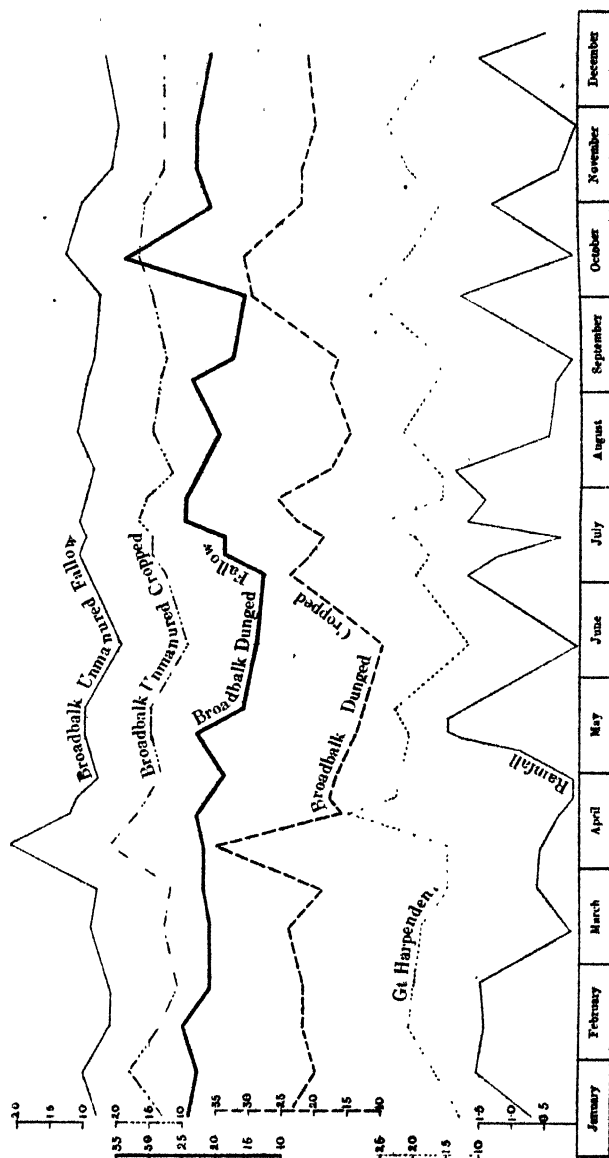
Fig. 6. Bacterial numbers in millions per gram for the various plots. The scale for each plot is given on the left-hand side. The rainfall is for the seven days preceding the determinations.

Turning to the connection between bacterial numbers and soil conditions, the most striking fact is the failure of temperature to cause a permanent rise in numbers. The usual result is that the numbers rise in spring when the soil temperature first rises, but they then fall (Fig. 6). There are subsequent variations, but neither the base line nor the average are much above the winter line, and on the Broadbalk dunged plots they fall below. This is completely different from plant growth, which in the main follows soil temperature. The relationship is not accidental, it has been obtained in the laboratory experiments here[1]. Conn[2] has obtained a similar result in the United States, the winter level being higher than the summer: he explains it by assuming that two sets of bacteria exist,--a summer and a winter set,—the latter being more numerous than the former.

It is possible that the flora undergoes seasonal variations, but even if it does, this does not explain the whole of the facts, for Russell and Hutchinson showed that the anomaly ceased as soon as the soils are partially sterilised; increases in the temperature then caused increases in bacterial numbers. Their hypothesis of a complex soil population seems more adequate. Löhnis and Smith[3] have suggested that these fluctuations may be due to life cycles, some steps in the cycle being countable, but not others. As the cycles are not worked out it is difficult to ascertain how far this new hypothesis meets the facts.

The moisture curve shows only a general kind of relationship with the biochemical activity, but it affords some explanation of the sluggish period. During the period of active accumulation the moisture on the dunged plot fluctuates between 18 and 21 %; during the sluggish period it is only 15 to 18 %. The connection is not altogether close because for a period in May the soil moisture rises to 20 %, but there is no corresponding rise in nitrate accumulation. But in the main active nitrification only occurs when some 18 % of water is present.

The low period following the spring rise does not fit in with any of the recorded data, nor does the high period in autumn when rain first comes after a time of drought; the drop in the first case and the rise in the second seems more than the conditions require. These phenomena, however, are exactly parallel to those observed by Russell and Hutchinson in their studies of partial sterilisation, and no doubt are due to the same cause. It is not possible to discuss these fluctuations in

[1] See E. J. Russell and H. B. Hutchinson, this *Journal*, 1913, **5**, 157 *et seq.*

[2] Conn, H. J., *Centr. Bakt. Par.* 1910, Abt. II. **28**, 422–434.

[3] Löhnis and Smith, *Journ. Agric. Research*, 1916, **6**, 675–702.
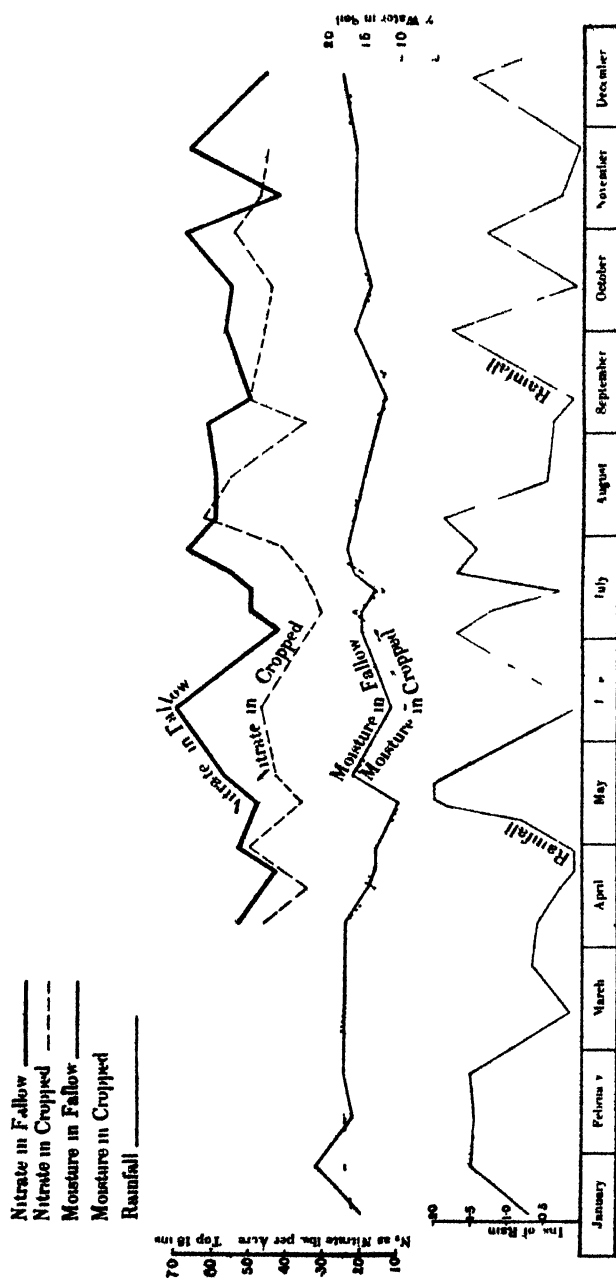
Fig. 7 Nitrate in the Broadbalk unmanured plots showing the effect of the crop

Fig 8   Nitrate in the Broadbalk dunged plots showing the effect of the crop

bacterial numbers more fully until the other organisms have been studied in more detail.   Investigations are now going on in our laboratory to discover means of counting the protozoa of the soil.

We must now turn to a closer discussion of the nitrate curves.

The difference between cropped and fallow land is sharply shown in the nitrate content of the soils.   On the fallow land receiving farmyard manure (Fig. 8) the nitrate steadily accumulates from April right up to September, and then drops to the winter level in January. The highest point reached on the dunged plot is no less than 170 lbs. nitric nitrogen per acre in the top 18 inches ; an enormous amount and representing a gain of 100 lbs. over the winter level.   The added dung only contains 200 lbs. nitrogen per acre.

The cropped land at first contains similar amounts, but as soon as plant growth becomes active nitrate ceases to accumulate, no more than 40 lbs. being present,- 90 lbs. less than in the fallow land at the same date, and 120 lbs. less than the maximum there.

This enormous difference in nitrate content between cropped and fallow land has been known for many years.   "The way," says Bacon[1], "to hasten the breeding of Saltpeter is to forbid the Sun and the growth of vegetables.   And therefore if you make a large Hovel, thatched, over some quantity of ground ; nay, if you do but plank the ground over, it will breed Saltpeter."

The difference is due to the crop but apparently some other action is concerned besides absorption of nitrates : if the amount of nitrogen in the crop (which presumably is equal to the nitric nitrogen absorbed) is added to the amount of nitric nitrogen in the soil the total falls short of the amount found in the fallow land.

The differences are only small and in the case of the Broadbalk unmanured plot they are within the error of experiment, but the persistence with which they appear in the other cases indicates that they are real.   The 1912 result is abnormally high because the crop was exceedingly poor, and in such circumstances weeds are always troublesome.   The phenomenon has been discussed in the earlier paper[2] where instances are given from the work of other investigators : it can only be satisfactorily studied in the laboratory, and we hope to be able to do this.   The results are:

[1] *Sylva Sylvarum*, p. 123.

[2] See this *Journal*, 1914, 6, p. 34.   An arithmetical error has crept into the September figures on p. 36, which vitiates the numerical results given there and also the top paragraph on p. 37 but does not affect this general conclusion.

	Hoos unmanured wheat plots		Broadbalk wheat plots, 1915				Maximum quantities. found
			Unmanured		Dunged		
	June, 1911	1912	July 26	Aug. 17	July 26	Aug. 17	
Nitrogen in crop*, lbs. per acre	22·6	6·1	21	21	70	70	70
Nitric nitrogen in soil, top 18″, lbs. per acre	15 3	12·b	44	40	37	69	85†
, Total, lbs. per acre	37·9	18 9	65	61	107	139	155
Nitric nitrogen in fal low land, top 18″, lbs per acre	53 7	46·1	70	62	124	150	170‡
Excess on fallow land, lbs. per acre ...	15·8	27·2	5	1	17	11	15

* The nitrogen in the crop is obtained as follows:

	Unmanured plot, per acre			Dunged plot, per acre		
	Dry matter	Nitrogen in dry matter		Dry matter	Nitrogen in dry matter	
1915	lbs. per acre	per cent.	lbs. per acre	lbs. per acre	per cent.	lbs. per acre
Wheat, grain... ...	859	1·899	16·3	2393	2·156	51·6
Wheat, straw ...	1305	0·392	5·1	4273	0·426	18·2
	2164	—	21 4	6666	—	69·8

† On Oct. 13th.   ‡ On Sept. 10th.

In the preceding discussion we have regarded the curves as pro-
duction curves. They are not, however, entirely production curves,
because losses are always going on; they only represent the balance
between gains and losses. The upward parts of the curve show that
production is predominating, but it does not necessarily represent the
whole of the production; there is always the possibility of loss. The
downward portions of the curve show that the losses are predominating,
but, again, they do not necessarily show the whole loss, because there
may be some production all the time. The curves, therefore, tend to
be flatter than if they were solely production curves.

We must now discuss the loss factors to see what light they throw
on the curves. Two causes are known for the loss of nitrate—leaching,
and absorption by the crop. On the fallow ground there is no crop,
and leaching is the only known cause at work. It remains to discover
whether this is sufficient to account for all the observed losses.

The kinks in the nitrate curve for the dunged fallow plot during May
and at the end of July and the beginning of August correspond with
periods at high percolation as shown by the drain gauges (Fig. 9). It is
not necessary to invoke any other cause to account for these, and right up

to September the measurements reveal no other factor except leaching which would cause loss of nitrate from uncropped soils. It should be noted, however, that only the drain gauges show this relationship, and
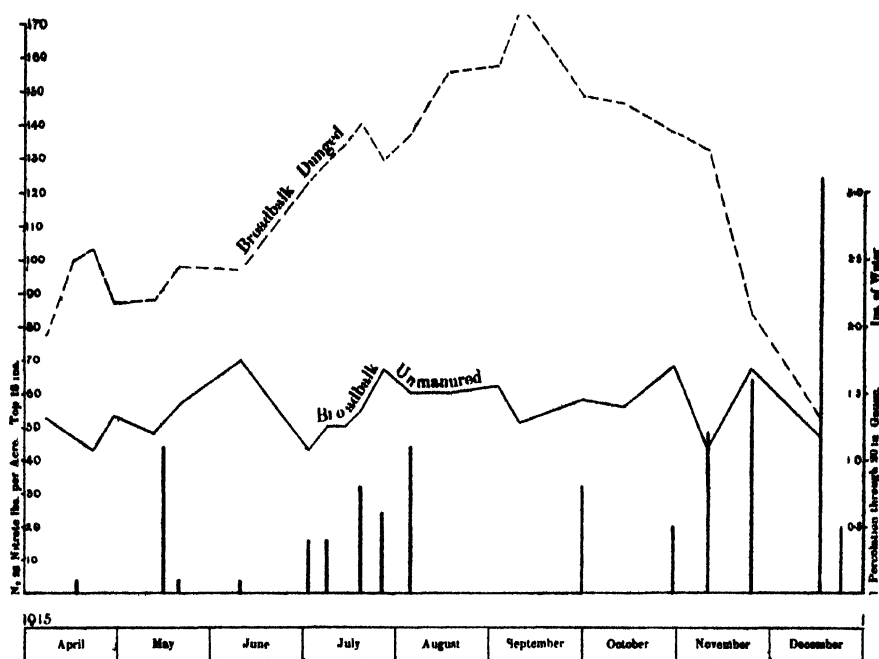
Fig. 9. Nitrate on the Broadbalk fallow plots, and amount of drainage water percolating through the 20″ drain gauge, this being indicated by the columns.

not the drains under the actual plots. The number of days on which these ran is considerably lower than those on which the gauges ran as shown by the following table:

1915	Rainfall (inches)	Drain gauges, percolation through 20″ gauge in inches	Drains in Broadbalk field, number of days on which they ran	
			Dunged plot	Unmanured
January ...	1·95	1·57	1	3
February ...	4 54	3·94	–	4
March ...	1·38	0·62	–	–
April ...	1·22	0·13	–	–
May ...	2·48	1·22	–	–
June ...	1·79	0·36	–	–
July ...	4·72	1·84	–	–
August ...	2·59	1·17	–	–
September	2·49	0·82	–	1
October ...	2·60	1·45	–	–
November	2·38	1·93	1	3
December...	5·56	5·32	1	9
Total ...	33·70	20·37	3	20

The drains on the dunged plot ran much less frequently than those on the unmanured plot, the large amount of organic matter having the effect of lightening the soil and facilitating percolation.

The sharp drop in nitrate content of the soil during September is difficult to explain solely as a leaching effect. During the whole month only 0·82 inch of drainage water passed through the 20-inch gauge, and yet 24 lbs. of nitrogen as nitrate is lost. Later on the percolation becomes very considerable, and the losses proportionately heavy. We have already dealt with this aspect of the question in our earlier papers[1]. All these losses are readily explicable as percolation effects excepting only that in September, which seems to indicate that when the nitrate becomes very high in amount some other source of loss may set in.

The changes on the unmanured plot are smaller: there is, however, a drop in June which is difficult to attribute to percolation.

Carbon dioxide. The formation of carbon dioxide is effected not only by the soil organisms proper, but also by plants, so that it is a more complex phenomenon than nitrification. The simplest case is seen on the dunged fallow plot where the crop is eliminated, but even this is not quite simple as a disturbing factor is introduced in the cultivation necessary to keep down weeds. Beyond the rise to a maximum in July and a fall to October the CO_2 curve in itself shows very little; it is, however, interesting in relation to the curve for bacterial numbers, because it reflects most of the fluctuations there (Fig. 2). The unmanured fallow is throughout on a lower scale but shows less connection with bacterial numbers: the fluctuations here, however, are probably too small to be significant (Fig. 1).

The effect of the crop is to be seen in May as one would expect from its rapid growth, and, curiously enough, in August at the time of ripening; in both cases the outpouring of CO_2 is sudden and extensive, so that the amounts rise to 1·6 and 1·9 %. It has already been pointed out that the bacteria fall to their lowest numbers at these points, and for a period there is no accumulation of nitrate; whether this inertness is due to the CO_2 or to some action on the part of the crop is not shown by field observations. The great increase in production of CO_2 in the soil at the time of ripening is not easily explained as a respiration effect; the roots at this stage are usually supposed to have ceased functioning. It may result from a decomposition of dead rootlets, as it is chiefly observed when the conditions are favourable for bacterial activity. The problem requires greater investigation.

[1] See note 2, p. 400.

Prior to May and after the crop is removed the CO_2 fluctuates in much the same way as the bacterial numbers.

The ordinary quantities of nitrate, CO_2 and bacteria present in soils.

Our results enable us to draw up the following Table, which will probably be found helpful to teachers and others in visualising the conditions in the soil. The figures are derived from a consideration of all our results; they do not include abnormal extremes, but give a fair idea of the usual conditions on a heavy arable loam:

		Total nitrogen		Nitrogen as nitrate		Numbers of bacteria, millions per gram	CO_2 in soil air
		parts per million	lbs. per acre	parts per million	lbs. per acre (top 9″)		
No manure. Condition poor	Cropped	990	2500	5–12	12–30	7–12	0·2–0·6*
	Fallow			8–15	20–36	7–12	0·2–0·4
Farmyard manure applied. Condition good	Cropped	2200	5000	10–20	25–50	15–25	0 5–1·0†
	Fallow			20–35	50–84	15–25	0·2–0·6
Ordinary arable field	Cropped	1500	3700	10–15	25–36	15–25	0·5–1·0‡

* Running on occasions up to 1·8.   † Occasionally up to 2 3.

‡ Occasionally up to 2 5.

Effect of ploughing on bacterial activity.

We took observations before and after ploughing but they throw less light than might have been expected on the effect of this important operation on the biochemical processes in the soil: we do not therefore propose to discuss them in detail.

The ploughing of the summer fallows was followed by a fall in bacterial numbers, while the autumn ploughing of the cropped land was followed by a rise. It is possible that the phenomena are related, but we prefer to leave the matter open for the present.

There was a distinct accumulation of nitrates on some of the plots after ploughing, but as a rule this happened so long afterwards as to make it uncertain whether there is any casual connection. Where the rise was pronounced it had usually followed an earlier rise in bacterial numbers with which it might at least equally probably be associated.

Our results are:

Field	Nitrate before ploughing*	Date of ploughing, 1915		Nitrate after ploughing					
Broadbalk, fallow ⎫	18	May 12	June 10	20	July 14	28	—	—	
dunged ... ...⎭	27	July 26	Aug. 6	28	Aug. 17	32	Sept. 6	33	
Broadbalk, fallow ⎫	12	May 17	June 10	14	July 14	10	—	—	
unmanured ...⎭	14	July 26	Aug. 6	12	Aug. 17	12	Sept. 3	12	
Great Harpenden ...	9	Sept. 10	Oct. 1	10	Oct. 29	13	—	—	

* N as nitrate, parts per million, mean of surface and subsoil contents.

Toulaikoff[1] working on the black earths of Samara in the semi-arid region of Russia has obtained a much sharper connection between nitrate accumulation and ploughing. His results are:

Nitric Nitrogen in different Fallows: parts of N per million of dry soil. (Five years' averages.)

	April		May		June		July		Aug.	Sept.	Oct.	Nov.
1st, 25 cms. (10 ins.)	Beginning	End	Beginning	End	Beginning	End	Beginning	End				
Black fallow	4	7	9	10	12	15	20	22	20	10	9	6
April fallow ...	4	5	9	11	11	14	17	18	13	13	8	6
May fallow ...	4	5	5	5	7	14	18	14	11	8	9	7
June fallow ...	4	5	5	3	5	4	6	8	9	4	6	5
2nd, 25 cms.												
Black fallow	5	4	4	5	8	8	7	8	9	6	6	4
April fallow ...	4	5	4	5	7	6	7	7	11	6	4	3
May fallow ...	3	3	4	4	4	4	5	8	9	8	7	6
June fallow ...	4	4	4	4	4	1	2	3	4	3	3	2

The black fallow had been ploughed in autumn, while the April, May and June fallows were ploughed about the 20th of their respective months. It will be observed that the nitrates show little sign of change till after the ploughing but they then rise rapidly.

It is interesting to note that Toulaikoff's figures are of the same order as ours but lower: in his case, however, the addition of dung did not raise the nitrates while in ours it causes a great increase.

[1] Toulaikoff, N. M. (Director, Besentchuck Agricultural Experimental Station), in *Selskoye Khosiaistvo Liesovodstvo*, 1915, **247**, 35–65 (abstract in *Bull. Int. Inst. Rome*, 1915, **6**, 797). Toulaikoff's figures are given as mgms. N_2O_5 per kg. of dry soil, but we have converted them to parts of N per million.

The mechanical analysis, specific gravity, and other data for the soils are given in this *Journal*, 1915, **7**, 44.

The methods of sampling and of analysis are the same as those described in the earlier paper.

The determinations have in all cases been made at intervals of about 10 days but more frequently during critical periods. The results are then plotted, and the points joined to give the curves here shown. This method has the drawback that it gives the appearance of a continuous record which, however, has not been attempted; on the other hand it is so much more convenient than the alternative and more correct method of drawing columns to indicate the separate points that we consider its advantages on the whole to out-weigh the disadvantages.

The data are given in Table I, and the curves in Figs. 1–5, but the main fluctuations may be briefly indicated.

Broadbalk Unmanured Fallow (Fig. 1).

This represents the simplest case; it is part of the Broadbalk unmanured plot which instead of being under crop as usual was this year (1915) divided into two parts, one cropped, and the other fallowed. No manure has been added since 1839, and nothing but wheat has been grown since 1843, so that the land is about as exhausted as possible.

The winter level for bacterial numbers is about 6 to 9 millions per gram; when the temperature rises in April this runs up to 21 millions. But it speedily falls, and during May is about 10 millions and in June below five—the lowest point of the year. Throughout July, August, and September the numbers are approximate constant about 10, then comes a rise in October, and a drop to the winter level in November and December.

The CO_2 in the soil air begins in the same way; the winter level is 0·1 to 0·15 %: then comes a rise in April to over 0·25 %, near which it keeps till May 17th, when the land was ploughed and a fall set in. During July there is a considerable rise to 0·45 % in August followed by a fall to the minimum in October, and then a rise to 0·2 % in November and December, when a new crop was beginning to grow.

The nitrates were not satisfactorily determined during the winter months so that the first record is in April at the rather high point of over 50 lbs. per acre, then comes a fall, and then a rise till June when a sharp drop sets in to 42 lbs. per acre, and again a rise to 67 lbs., about

which the quantity fluctuates till November and December when it falls. There is no accumulation during the autumn as on the dunged. fallow: 60 to 70 lbs. seems to be the maximum obtainable and once this is reached little further action takes place.

We are much surprised at this high value for so very exhausted a soil, but we have obtained similar ones before. At no time this year did we find less than 30 lbs. of nitric nitrogen per acre in the top 18 inches, and 15 lbs. in the top 9 inches; i.e. 7 or 8 parts per million of soil. In the preceding paper we show that the similar Hoos Field unmanured plots contained on the fallow part up to 13 parts of nitric nitrogen per million, or 54 lbs. per acre in the top 18 inches, and on the cropped part up to 9 parts per million, or 23 lbs. in the top 9 inches. Now we know from the Broadbalk Plot 10 that the unmanured crop responds to more nitrate, yet it leaves all this quantity untouched. Of course the roots could hardly be expected to exhaust the soil because, as the grass plot experiments show, diffusion is an exceedingly slow process, and the roots only search through a relatively small part of the soil. But one would look for a larger utilisation than this.

That exhausted soils can still go on producing nitrate is shown by the amount of nitrate in the drainage water from the drain gauges which after 46 years of bare fallow still yield some 35 lbs. of nitric nitrogen per acre from 20 inches of soil; this can only be part of the nitric nitrogen present because the amount of percolation is never sufficient to wash it all out. It is possible that the method brings out something besides nitrate, but such comparisons as have been made in our laboratories with colorimetric methods are against this view. And, moreover, light soils give the low values that would be expected.

In comparing the CO_2 and the nitrate with one another it must be remembered that a period of non-production of CO_2 must appear as a falling line because of the perpetual loss by diffusion. Further, this perpetual loss makes all the upward parts of the CO_2 curve less steep than they would otherwise be. The fluctuations in nitrate are about a month later than those in bacterial numbers.

Broadbalk Dunged Fallow (Fig. 2).

This is part of the plot which has received 14 tons of dung annually since 1843; it contains in comparison with the unmanured:

	Dunged	Unmanured
Organic matter (loss on ignition) ...	10·0.%	4·3 %
Nitrogen	0·20	0·10

The richness in organic matter is reflected in the numbers of bacteria which during the winter season remain at some 22 millions, about three times as high as the unmanured plot. There is no rise in April or May when the temperature rises, but a very pronounced fall to 13 millions in June, then a quick rise in July, followed again by a drop throughout August and September with only one break: then comes a rapid rise in October to 34 millions, the highest point reached, and finally a quick drop to the winter level.

The amount of CO_2 in the soil air starts from its winter level of about 0·1 % and rises gradually to 0·36 % in May, but there is no rapid rise as is recorded for the nitrate during April. There is a kink in the curve in May when the land was ploughed, and afterwards a slow rise to a maximum (0·63 %) in July, the land was then again ploughed and there was a continuous drop; nothing was obtained to resemble the continuous rise in the nitrate curve; the ploughing, however, greatly facilitated the escape of CO_2. This apparent cessation of activity in July is very remarkable, and is not easily explained as a mere temperature or moisture effect; the soil is moist and warm, yet both bacterial numbers and CO_2 remain low. Nitrate is steadily accumulating, however.

The CO_2 differs but little from that on the unmanured plot. This unexpected result is due in part to the great difference in physical texture, the unmanured soil being close and compact and not readily favouring diffusion, while the dunged soil is much more porous.

The nitric nitrogen begins at 80 lbs. per acre in top 18 inches in April and rises rapidly to over 100, then it drops till May, when a steady rise begins which, with only one break in July, is continued to September, when the extraordinarily high quantity of 170 lbs. of nitric nitrogen per acre is present. Beyond this point there comes a rapid drop to the winter level. During this fall more than 100 lbs. of nitrogen are lost. Yet through the whole period of loss the drains only ran on one day. The result suggests that something besides leaching is involved (Fig. 9).

Excepting during June the fluctuations in bacterial numbers are reflected in those of the CO_2 quantities; the high rise in October, however, does not lead to a corresponding rise in CO_2. The nitrate curve falls out of line, only till June does it resemble the CO_2, its further rise till September being shown neither by the bacterial numbers nor by the CO_2.

Broadbalk Unmanured Cropped (Fig. 3).

The bacterial numbers fluctuate as on the fallow land: starting from the winter level of about 7 millions per gram they rise in April to 16, then fall to less than 5 in June; they rise and remain about 10 millions with some fluctuations, and rise in October, finally falling to the winter level in November.

The CO_2 curve differs considerably. The winter level is 0·2 % : a rise sets in in March to 0·6 % at the end of April, then comes a drop in May, and a rise again to 0·6 % beyond which there is no further rise till July, when the amount rapidly rises to over 1·8 % followed by a sudden drop to 0·2 % in September and October, and a small rise in November.

The nitrate curve rises and falls with the CO_2 curve, the maximum amount, 55 lbs. per acre of nitric nitrogen, being attained in October. The curve also resembles that for bacterial numbers, but it is about three weeks to a month behind.

The difference in nitrate content between cropped and fallow land is much less on the unmanured than on the dunged plots, and is never more than 26 lbs. per acre in the top 18 inches. The crop contained 21 lbs., so that the cropped land seems to have contained as much nitrate as the fallowed land (Fig. 7).

Broadbalk Dunged Cropped (Fig. 4).

The bacterial numbers fluctuate more than on the fallow plot but in the same general way. The winter level is, as on the fallow plot, about 22 millions, but in April the numbers run rapidly up to 35 millions, followed by a drop to 10 millions in June: then comes a rise to 26 millions in July: a drop in August and early September, but a sharp rise to 31 millions in October, finally a fall to the winter level. It is remarkable that the March numbers rise so much higher than those of the fallow plots.

From April onwards there is a close agreement between bacterial numbers and rainfall curves excepting only in early May and early August, the times when the CO_2 is at a maximum. These curves represent the amount of rain falling during the seven days preceding the observations.

The CO_2 curve begins at a low winter level of 0·4 %, rises steadily then finally sharply to 0·8 % in April: then comes a fall in May, followed by a rapid and considerable rise to 1·6 % : then comes a fall

in June and July, and a very marked rise to 1·9 % in August. · After this there is a sudden fall to 0·3 % in September and October, followed by a rise to 0·5 % in November.

The nitrate curve shows the same fluctuations as the CO_2 curve till June, and again from early August onwards. It rises sharply in April to 88 lbs. per acre of nitric nitrogen in the top 18 inches, then falls to 66 lbs., rises to 75 lbs., drops and rises, and then falls in two abrupt steps to 38 lbs. in July. Then it rises to 72 lbs. in August, keeps constant in September, rises in October to nearly 90 lbs. and falls from this level in November. The maximum difference between the cropped and the fallow plot is in September, when the fallow contained 170 lbs. and the cropped only 70, a difference of 100 lbs. per acre in the top 18 inches. By December all this difference has been lost and both have fallen to the same level (Fig. 8).

In previous years the highest quantity of nitric nitrogen recorded in parts per million had been 18: this year the peaks on the curve are at 20 although in one case we found 25.

As before, the nitrate curve is like that for bacterial numbers but about three weeks later: the exception is at the time of the very high CO_2 content when the numbers are low.

Great Harpenden Field (Fig. 5).

In general condition this comes between the Broadbalk unmanured and the Broadbalk dunged plots: it is farmed in the ordinary way; it carried potatoes in 1913 when it was dressed with dung and artificials, wheat in 1914 without manure, and oats in 1915 ·with a spring dressing of 1 cwt. sulphate of ammonia and 2 cwts. superphosphate per acre. Observations were made here to see how far the relationships observed on the Broadbalk plots would be likely to hold under ordinary soil conditions.

In the main the fluctuations are very similar to those of the Broadbalk plots: as compared with the dunged plot, there are the same rises in April and May, and the same violent outpouring of CO_2 in July and August. Up till July the curves are flatter than on the Broadbalk dunged plot, and by themselves do not show so close a relationship with the rainfall curves. But when placed alongside of the Broadbalk curves it becomes obvious that the same relationships with rainfall exist but they are less pronounced.

On the Broadbalk unmanured plots the flattening out process begun

on the Harpenden Field curves has gone further so that some of the rises in the dunged plots become falls here, and *vice versa*.

The bacterial numbers in winter time lie between 15 and 20 millions: in April they rise rapidly to 30 millions, the maximum for the year: then they drop to a minimum of 12 millions in June: they rise again in July, following the high rainfall: then once more they drop, and again rise at the end of September, also accompanying high rainfall, finally they drop again. These fluctuations are similar in general character to those on Broadbalk, and show the same initial connection with temperature and subsequent relation with rainfall.

The CO_2 to begin with follows the curves for bacterial numbers, but in May it shoots upwards to 1 %, and finally in August it becomes 2·5 % of the soil air. Then there is a fall to 0·3 % in October, and the fluctuations again follow the curves for bacterial numbers. Here, as in the other cases, there is a great outpouring of CO_2 in the soil at the time of ripening of the crop and simultaneously a depression of bacterial numbers although the rainfall curve would have indicated a rise.

The nitrate begins in April at 50 lbs. per acre in the top 18 inches: towards the end of the month it rises suddenly to 67 lbs. just as on the Broadbalk unmanured plot: then comes a fall, and in June a great rise to 94 lbs. followed by a rapid drop to the minimum, 35 lbs., after which a rise sets in, then a fall, then after ploughing another rise, finally after October a fall to 40 lbs. per acre in the top 18 inches. The curve differs from the Broadbalk dunged plot mainly in that it rises so much higher early in July, which may be attributed partly if not wholly to the dressing of sulphate of ammonia.

The rate of nitrification of sulphate of ammonia in the soil.

We had hoped to obtain definite information on this point from these observations, but we were disappointed. The effect of the sulphate of ammonia is not at all clear. It was applied on April 5th and the amount used (1 cwt. per acre) contained 22 lbs. of nitrogen. Twenty-four days after the application the nitric nitrogen in the soil rapidly gained 9 lbs. which might be attributed to the added ammonia except for the circumstance that the Broadbalk unmanured plot showed a similar rise. The only period when Harpenden field falls out of line with the rest is as already stated in early July. It is difficult to believe that the ammonia had so long remained unnitrified if it had been within the range of action of the bacteria. The explanation may be that it

only slowly mingles with the soil by reason of the high degree with which it is absorbed.   There is remarkably little evidence as to the rate of nitrification of sulphate of ammonia in field soils, and more measurements are needed.

The Rothamsted evidence is obtained from the analysis of the drainage water of Broadbalk field and is in some respects more satisfactory than can be derived from direct field determinations.   The analyses have not been made as systematically as in the case of the drain gauges because of the difficulties of sampling, but sufficient data have accumulated to bring out the general facts.

The composition of the drainage water from Plots 8 and 10 in Broadbalk Wheat Field before and after the application of ammonium salts was as follows:

Nitrogen as nitrates per million of drainage.

	Before application, Jan. 9th	After application on Feb. 23	
		March 1st	April 26
Plot 8	10·2	14·1	—
Plot 10	9·1	15·2	35·2

Plot 8 receives complete mineral fertilisers in addition to ammonium salts, while Plot 10 receives in addition superphosphate only and no potash.   Here the marked increase is observed some weeks after the application, but as the drains had not been running in the meantime there is no way of telling at what period between March 1st and April 26th it took place.

In the autumn measurements the interval is less though it is not clear whether this is due to more rapid action (the soil is certainly warmer) or to more rapid detection because the drains ran at a shorter interval.   A small increase in nitrate has been detected only 40 hours after the application of the ammonium salts, but the marked increase did not come for three weeks.

The salts added are a mixture of ammonium sulphate and ammonium chloride, and the large amount of chloride on Oct. 27th shows that washing out had already begun, so that any nitrate formed could have been detected.

Laboratory experiments have been made both by Schloesing and at Rothamsted and have shown that action may be practically completed in 8 to 15 days under favourable conditions.

Composition of the Drainage Water in the Rothamsted Wheat Field before and after the Application of Ammonium Salts.

	Plot 15. October—November, 1880				Plot 7. October—November, 1		
	Date	Chlorine	Nitrogen as ammonia	Nitrogen as nitrate	Date	Chlorine	Nitrogen as nitrate
Before application of ammonium salts...	Oct. 10	22·7	None	8·2	Oct. 23	19·3	5·1
	—	—	—	—	„ 27	28·2	5·6
Date of application	„ 25	—	—	—	„ 29–30	—	—
After application of ammonium salts...	„ 27, 6.30 a.m.	146·4	9·0	13·5	Nov. 11, 7 a.m.	50·6	32·5
	„ „ 1 p.m.	116·6	6·5	12·9	„ „ 10 a.m.	42·0	29·5
	„ 28	95·3	2·5	16·7	„ „ 1 p.m.	41 2	28·2
	„ 29	80·8	1·5	16·9	„ „ 5 p.m	39·9	26·0
	Nov. 15 and 16	54·2	None	50·8	„ 13, 7 a.m.	28·0	20·5
	—	—	—	—	„ „ 10 a.m	29·9	20·2
	—	—	—	—	„ „ 1 p.m.	28·5	15·9
	—	—	—	—	„ „ 5 p.m.	26·6	13·3
	—	—	—	—	„ 15, 1 p.m.	23·5	16·5
	—	—	—	—	„ „ 3 p.m.	26·4	16·8
	—	—	—	—	„ „ 5 p.m.	26·1	16·3

CONCLUSIONS.

The changes in bacterial numbers and in nitrate content of the soil and in CO_2 content of the soil air have been determined at frequent and regular intervals during several seasons on five different plots of land, and the results have been set out on curves.

1. There is sufficient resemblance between the curves for bacterial numbers, CO_2 (except for a period on cropped land), and nitrate to justify the conclusion that they are all related.

2. The curve for nitrate, however, is always behind that for bacterial numbers, the lag amounting to two or three weeks. Assuming, as we think we may, that the curves are connected, this would indicate two stages in nitrate production: one related to the bacterial numbers, the other not. We bring evidence against the view that the stages are simply ammonia production and then nitrate production: the division has apparently to he carried further back, and ammonia production divided into two stages.

3. The biochemical decompositions in the soil are determined in the first instance by the temperature, and do not proceed to any notable extent below 5° C.

4. As soon as the temperature rises action begins rapidly. But it soon slows down and other factors begin to operate.

EXPERIMENTAL DETAILS.

*(The results from Dec 19th, 1912, to Sept 21st, 1914, are given in the previous paper, this Journal, 1915, **7**, 1—48.)*

Date 1915	Broadbalk Fallow Plots								Broadbalk Cropped Plots								Harpenden Field			
	Unmanured				Dunged				Unmanured				Dunged							
	CO_2 in soil air per cent	N as Nitrate parts per million	Bacterial numbers millions per grm	Water per cent	CO_2 in soil air per cent	N as Nitrate parts per million	Bacterial numbers millions per grm	Water per cent	CO_2 in soil air per cent	N as Nitrate parts per million	Bacterial numbers millions per grm	Water per cent	CO_2 in soil air per cent	N as Nitrate parts per million	Bacterial numbers millions per grm	Water per cent	CO_2 in soil air per cent	N as Nitrate parts per million	Bacterial numbers millions per grm	Water per cent
Jan 12	0·08	—	8	15	0·07	—	24	22	0·16	—	8	16	0·36	—	24	22	0·50	—	12·5	20
„ 26	0·12	—	10	21	0·13	—	23	22	0·21	—	13	17	0·32	—	20	21	0·38	—	17	19
Feb. 10	0·07	—	6	16	0·11	—	25	22	0·15	—	9	17	0·18	—	22	23	0·35	—	21	18
„ 23	0·15	—	6	17	0·15	—	20·5	18	0·21	—	6	18	0·32	—	22	22	0·53	—	20·5	18
March 11	0·15	—	9	16·5	0·14	—	20·5	22	0·21	—	8	16·5	0·44	—	24	21·5	0·44	—	18·5	18·5
„ 23	0·15	—	7	17	0·16	—	22	24	0·31	—	7	17	0·42	—	19	22·5	0·47	—	15	20
April 7	0·26	11	21	17·5	0·17	21	22	21	0·40	9	16	16·5	0·60	15	35	22	0·58	14	15	18
„ 17	0·20	9	12	15·5	0·22	23	23	19	0·39	7	12	13	0·86	22	16	20	0·79	12	30	16
„ 22	0·23	11	11	12·5	0·26	33	23	19	0·38	9	9	18	0·78	19	18	19	0·62	15	23	15
„ 29	0·25	12	8	13	0·25	25	18	17	0·63	12	8·5	12	0·77	20	17	16	0·66	15	22	15
May 12	0·28	10	10	10	0·36	23	23	15	0·49	7	10	10	0·72	11	14	12	0·70	11	21	11
„ 15	0·22	—	—	—	0·24	—	—	—	0·36	—	—	—	1·01	—	—	—	0·56	—	—	—
„ 17	0·19	—	—	—	0·35	—	—	—	0·45	—	—	—	1·40	—	—	—	0·91	—	—	—
„ 20	0·19	14	10	16	0·31	24	16	20	0·56	10	10	16	1·62	19	—	20	1·06	14·5	23	18
June 10	0·25	12	4·5	11	0·39	30	14	15	0·67	12	4·5	9	0·67	13	10	10	1·03	14	12	19

The following table is set sideways on the page. Each "Nitrate" value is a decimal; the accompanying "Water" values are integers, with the upper figure referring to surface soil (top 9″) and the lower figure to subsoil (2nd 9″).

Date (1915)	Nitrate	Water		Nitrate	Water		Nitrate	Water		Nitrate	Water		Nitrate	Water	
July 2 …	0·20	10	10	0·50	27	13	0·61	7	8	0·97	16	24	1·12	17	20
,, 8 …	0·28	8	11	0·51	15	19	0·68	7	10	0·49	10	21	0·88	22	18
,, 14 …	—	13	10	—	41	19	—	8	10	—	17	19	—	12	20
,, 19 …	0·36	8	11	0·63	14	25	1·25	5	12	1·17	8	23	1·77	8	25
,, 26 …	—	15	10	—	41	25	—	8	11	—	15	26	—	13	16
Aug. 6 …	0·45	6	9	0·58	16	liquefied	1·83	8	7	1·49	10	18	2·51	7	13
,, 17 …	0·32	12	11·5	0·51	36	20	1·61	8	10	1·86	14	15	2·32	11	18
Sept. 3 …	0·19	11	10	0·43	23	24	0·91	9	9	1·12	10	18	1·36	8	16
,, 10 …	0·29	15	9	0·33	28	18	0·19	8	8	0·33	6	17	1·08	8	15
Oct. 1 …	0·14	13	8	0·22	26	16	0·18	11	6	0·21	13	30	0·28	13	20
,, 13 …	0·12	13	13	0·29	25	34	0·17	7	10	0·30	8	31	0·23	7	19
,, 29 …	0·17	12·5	10·5	0·20	32	22	0·11	11	12	0·24	15	22	0·17	18	20
Nov. 10 …	0·23	11	6	0·32	40	23	0·30	8	11	0·39	15	22	—	9	18
,, 24 …	0·23	12	5	0·35	25	23	0·26	8	8	0·53	18	20	0·35	12	23
Dec. 16 …	—	13	7	—	22	21	—	8	8	—	16	21	—	8	19
,, 23 …	0·20	7	—	0·26	12	—	0·26	12	—	0·46	13	—	0·38	5	—
1916															
Jan. 13 …	—	9	7	—	11	24	—	9	7	·	13	22	—	11	14
,, 24 …	0·20	9	7·5	0·40	10	20	0·23	9	8	0·52	10	23	0·22	8	16
Feb. 10 …	—	7	6	—	14	15	—	10	6	—	13	12	—	7	11

Notes. In the "Nitrate" and "Water" columns the upper figures refer to surface soil (top 9″) and the lower figures to subsoil (2nd 9″). To convert Nitrate, parts per million, into Nitrate, lbs. per acre, multiply by 2·6 in the case of Broadbalk unmanured plot and Harpenden field, and by 2·3 in the case of Broadbalk dunged plot.

METEOROLOGICAL DATA.

Date 1915	Soil temperature, °C (on plot)	Soil temperature, °C (self-recording thermometer)	Rainfall of preceding 7 days, inches	State of Ground	Weather on day of sampling	Weather on preceding day	Observations
Jan. 12	3·0	2·5	0·70	Very wet	Rained early; fine afterwards	Fine	Difficult to sample soil. Some gas samples bubbled through water at 6". Difficult to withdraw soil gas.
,, 26	2·3	3·0	1·54	Wet, but firm	Sun after mist	Calm and dull	
Feb. 10	3·2	4·0	1·45	Frozen on surface	Sunny and warm	Cold and showery	
,, 23	2·3	3·5	1·49	Very wet	Sun; cold N. wind	Rain in evening	Difficult to withdraw soil gas.
March 11	3·9	5·0	0·15	Somewhat drier	Dull but fine	Fine and dry	Little rain during sampling.
,, 23	5·0	6·0	0·62	Very wet and sticky	Mild and misty; some showers	Fine and dry; much rain in night	
April 7	6·3	8·0	0·64	Ground drying	Fine	0·4" rain fell in afternoon	More liquefying bacteria in this sample than in previous ones.
,, 17	6·8	8·7	0·30	Dry on surface; moist at 6"	Sunny; light N.E. breeze	Fine and dry	Great Harpenden Field has been harrowed.
,, 22	7·6	8·5	0·11	Very dry on surface; moist at 6"	Fine, dry and warm	Fine and dry	
,, 29	12·5	12·0	0·06	Very dry on surface; just moist at 6"	Hot; sun all day	Hottest day so far; sun all day	Harpenden Field has been rolled.
May 12	14·0	13·6	0·08	Very dry and hard	Dry; barometer low	Dry; barometer falling	Broadbalk dunged fallow ploughed to-day; but not unmanured fallow.
,, 15	10·8	9·7	1·95	Wet	Weather fine and sunny	Very heavy rain on May 13	Broadbalk unmanured fallow ploughed to-day.
,, 17	11·0	11·5	1·98		Showery and warm	Dull and damp; nght warm	
,, 20	11·5	12·2	1·88	Good	Dull; inclined to rain	Little rain in night	
June 10	11·5	12·2	0·05	Very hard and dry	Sultry and fine	Little rain after drought	Difficult to sample soil on account of dryness.
July 2	16·1	19·0	1·66	Very good condition	Very close and fine	Heavy thunder rain on June 30 (1·21"). July 1 rather dull; cool, then warmer	
,, 8	15·6	17·5	1·29	Good condition	Dull and calm	Much rain (·318")	Fallows have been harrowed down.
,, 14	14·6	18·2	0·36	Nicely moist	Fine		
,, 19		17·2	1·69		Dull	Sunny	
,, 26		17·8	1·45	Sticky	Calm, dry and sunny	Sunny in morn.; shower in aft.	Broadbalk fallows have been ploughed.
Aug. 6	11·5	17·3	1·86	Nice and moist	Sunny, dry and calm	Cloudy; sharp shower in aft.	
,, 17	16·0	19·5	0·46	Nice and moist	Brilliant sunshine; very close	Fine and dry after rain on 15th	Crops cut.
Sept. 3	11·8	13·0	0·44		Fine; slight showers. N.W. wind.	Dull and damp	Ploughing begun on Broadbalk Cropped plots after samples were taken.
,, 10	14·9	16·5	0·13	Very dry on surface	Sunny after heavy dew	Fine and dry	Great Harpenden Field ploughed after samples were taken.
Oct. 1	7·8	10·0	1·79	Good condition	Sunny	Cold and dull; no rain	Broadbalk Cropped land very rough.
,, 13	12·1	13·0	0·06	Nice condition	Sunny after heavy dew	Beautifully sunny	Broadbalk fallow has been harrowed.
,, 29	7·1	7·0	1·26	Very wet	Sunny after mist	Cold. squally (·164")	Dung ploughed in on Broadbalk.
Nov. 10	5·5	6·0	0·28	Rather sticky	Sunny; cloudy at times	Dull and rainy	Great Harpenden has been sown with oats and harrowed.
,, 24	2·8	3·5	0·05	...	Fair; N. winds; frosty at night	Overcast; frosty at night	Broadbalk has been drilled and harrowed.
Dec. 16			1·47	...	Mild and fair		
,, 23	5·0	5·0	0·55	...		Dull and showery	
1916 Jan. 13	—	—	0·34	Very wet	Mild, sunny after rain	Sunny, mild	Impossible to get gas out in some places
,, 24	5·9	5·9	0·52				

Note. The soil temperature on the plot is taken by inserting a glass thermometer; the self-recording thermometer is with the meteorological instruments and is buried 6" down in the soil.

5. Moisture is one of these. Action came to a minimum in June when the moisture fell to 10 % by weight of the unmanured soil, and 15 % by weight of the dunged soil, or 16 and 22 parts respectively by volume, assuming there was no contraction.

6. Rainfall is an even more important factor: a shower of rain having a notable effect in starting the decompositions. It seems probable that the dissolved oxygen is an important factor here.

7. The growing crop exerts a depressing effect, though whether by taking up the dissolved oxygen, by giving out CO_2, or by some other action, is not clear.

8. The fluctuations in bacterial numbers are not wholly explicable as functions of the temperature and moisture content. Some of the rises and falls are of the kind obtained during the investigations on partial sterilisation: further work on this problem is in hand in our laboratories.

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THE COMPARATIVE KEEPING QUALITIES OF PALM KERNEL, COCONUT, GROUND-NUT AND OTHER OIL-CAKES.

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ONE of the difficulties which hinder the extended use of the nut-oil-cakes (palm kernel, coconut and ground-nut) is the prevalent impression that these cakes deteriorate rapidly on keeping. In recent articles dealing with these cakes frequent reference has been made to this point. Thus Voelcker[9] in his Annual Report for 1914 states that "one inconvenience attaching to palm-nut and coconut cakes is that they do not keep as well as linseed and cotton cakes and that there is a tendency for them to turn rancid." In his Report for 1915, however, he says, "I have come across but few instances either with palm-nut cake or meal, in which these have been rancid or not in good condition." Murray[8] states that manufacturers should realise that they still have to reassure farmers regarding the keeping qualities of palm kernel cake. In a later article in the *Journal of the Board of Agriculture*[3] it is stated that "palm-nut kernel cakes in the past have had the reputation of soon going rancid....At the present day, before the kernels are crushed they are subjected to a process of cooking, by which the ferment that causes the oil to turn rancid is rendered inactive." The validity of this latter assertion seems doubtful in the light of the recent work of Calder[2] which shows that the lipase, present in the resting seed in the form of its zymogen, is not destroyed during the manufacture of the cake, the mass of crushed kernels not being sufficiently heated in every part prior to pressing to secure this object completely. Similar statements have been made as to the liability of coconut and ground-nut cakes to become rancid on storing[3, 4].

In view of these facts and of the endeavours which are being made to increase the use of these cakes in this country, it was thought advisable to re-examine the whole subject of the keeping qualities of these cakes and to compare them, in this respect, with other cakes, which are already widely used by British farmers. The three nut-cakes were therefore compared with linseed, undecorticated cottonseed, "soycot" and soya cakes, the cakes being kept

(a) under ordinary farm conditions in the cake store at The Manor Farm, Garforth;

(b) in the laboratory under conditions most likely to promote decomposition.

Of each particular kind of cake four adjacent cakes from the middle of a press were obtained fresh from the crushers. Of these four cakes, two were stored at Garforth in the cake store, stacked in the usual way alongside the cakes then being fed on the farm, whilst, of the other two, one was used for the laboratory trials and the other kept in reserve. When the cakes were sampled for analysis, strips about eight inches wide were taken from the middle of each cake, ground up, mixed separately and sampled by quartering in the usual way.

In addition to careful observation of the appearance, physical condition and other external characteristics of the cakes, the following points were examined:

(1) The changes in the composition of the cake with respect to (a) crude protein, (b) true protein, (c) oil, due allowance being made for the variation in the moisture content of the cake during storage.

(2) The increase in the amount of the free fatty acids in the oil during storage.

(3) The part played by the oil in any changes undergone by the nitrogenous constituents of the cake.

FARM TESTS.

The two cakes of each kind were put into the cake store on March 28th, 1916, the first cake being sampled on June 15th, and the second on September 13th, 1916, by the method described above. The samples were thus obtained, roughly after three and six months storage respectively. The six months storage covered the whole of the summer months, the cakes thus being kept at temperatures very suitable for the activity of micro-organisms. The percentage composition of the dry matter of the cakes at each stage is given in Table I.

At the end of the six months there was no sign of mould on any of the cakes nor was any unpleasant smell noticeable. The coconut cake had become quite soft and could be crumbled in the hand and was, therefore, difficult to handle. In view of the difficulty which Mackenzie and Powell(6) experienced in getting cattle to clean up coconut cake, even when it was mixed with cottonseed and linseed cakes, unless the coconut cake was in moderately big pieces, this softening would be an apparent objection to the lengthy storage of this cake. The palm kernel cake could be broken with moderate ease, whilst all the other cakes were still quite hard.

TABLE I. *Cakes stored at the farm.*

Kind of cake	Length of time stored	Percentages calculated on the dry matter			
		Crude protein	True protein	Oil	Free fatty acids as % of oleic acid in the oil
		%	%	%	%
Linseed	Nil	32 78	28 77	13 43	11·23
	3 months	31 19	28 07	13 46	18 07
	6 months	32 35	28 01	14 02	29 82
Ground nut	Nil	34 08	31 21	11 27	14 65
	3 months	31 98	28 19	10 72	37 65
	6 months	32 99	27 92	10 25	56 02
Coconut	Nil	20 68	19 92	15 59	5 84
	3 months	20 29	19 42	14 46	45 39
	6 months	21 21	19 95	12 55	73 45
Palm kernel	Nil	19 49	18 88	10 38	5 70
	3 months	19 00	18 51	10 68	25 34
	6 months	19 02	19 01	10 76	40 93
Undecorticated cottonseed	Nil	25 55	23 69	5 33	61 48
	3 months	25 03	22 93	5 77	82 85
	6 months	24 99	23 85	5 47	87 44
"Soycot"	Nil	34 66	33 87	6 01	20 95
	3 months	36 01	32 91	6 04	37 42
	6 months	36 07	33 44	5 85	65 33
Soya	Nil	47 94	46 35	6 61	5 18
	3 months	48 75	46 79	6 90	8 80
	6 months	48 41	46 52	7 22	16 37

It will be seen that, with the possible exception of coconut cake, the cakes show no change in the percentage composition of their dry matter, beyond the limits of error due to sampling and the possible slight variation between the cakes as manufactured, during the six months storage. The one noticeable change, common to all the cakes, though in varying degree, is the increase in the amount of free fatty acids present in the extracted oil. In this connection the most striking

feature is the high initial acidity in the case of the cottonseed cake, due to the fact, subsequently ascertained, that the cottonseed cakes used were made from a not very satisfactory sample of cottonseed from the notoriously bad crop of 1915. This explanation is borne out by the following data obtained from a number of samples of cottonseed cake examined very soon after their manufacture.

TABLE II. *Acidity of fresh Cottonseed Cake.*

Kind	Year of crop	No of samples	Oil (average) %	Free fatty acids expressed as % of oleic acid in the oil		
				Max	Min.	Average
Egyptian ..	1915	14	4 72	80 67*	11 44	34 71
Egyptian ...	1916	6	4 76	22 33	8·57	12 71
Bombay .	1915	2	4 79	12 11	7 46	9 78
S. American	1915	1	3 80	—	—	13 84
Soudanese ...	1915	1	4 27	-		7 32

* Described by the crushers as made from the oldest seed of a very poor crop

The final acidities, as shown in Table I, are all high, even the soya cake, which is the lowest, having an acidity more than three times the initial value. Leaving out of consideration the abnormal case of cottonseed cake the highest final acidity is shown by the coconut cake, which also shows the greatest relative increase, the lowest relative increase being given by linseed cake. If this final free acidity be taken as a measure of the keeping qualities of the cakes then, after soya and linseed cakes, palm kernel cake is the most satisfactory. These results would indicate that the presence of a fat-splitting enzyme is common to all the oil-cakes and cannot be adduced as a specific disadvantage of palm kernel cake.

It is difficult to say just what practical significance should be attributed to this increase in acidity. The presence of appreciable quantities of free fatty acids in a feeding-stuff is commonly regarded as undesirable but, provided the acidity be not so high as to cause actual derangement of the digestive functions, it is at least doubtful whether the nutritive value of the oil is seriously lowered by this liberation of the fatty acids present in it. Such direct evidence as is available points at least to this conclusion. Thus in experiments carried out by Munk(7) equally good results were obtained whether mixed fatty acids were fed as such or in the form of their glycerides, the results indicating that the animal body is capable of building up neutral fat from these free acids and depositing them in the body.

LABORATORY TESTS.

For the purpose of the laboratory experiments a strip from the middle of each cake was used. This was ground as finely as possible, a sample drawn for immediate analysis, and then lots of 200 grams of each cake taken and incubated under the following conditions:

Exp. 1. The ground cake was placed in a wide-mouthed bottle, 8 % of water was mixed in with it, the bottle loosely corked, and the whole incubated at 37° for twenty-six days.

Exp. 2. Similar to Exp. 1. The cakes were weighed before and after incubation.

Exp. 3. In addition to the 8 % of water, 5 c.c. of toluene were added to each cake in order to prevent mould formation.

It will be noted that, in each case, the cakes were kept in a confined space, in moist condition and at a relatively high temperature, conditions which may be regarded as likely to ensure a severe test of keeping properties.

During the periods of incubation in experiments (1) and (2) the first cake to show mould was the "soycot," which was distinctly mouldy at the end of two days. This was closely followed by cottonseed, ground-nut and soya cakes in the order named. At the end of four days the palm kernel and coconut cakes, though showing no signs of mould formation, had an ethereal and at the same time a somewhat cheesy smell. At the end of the period of incubation the four first-named cakes were mouldy throughout the whole mass, the "soycot" and soya cakes having a smell resembling that of fish meal. The linseed cake showed a development of mould at one spot but, apart from a slightly mouldy smell, no other change was apparent. Neither the palm kernel nor coconut cakes showed any sign of mould formation, but the cheesy smell was intensified in the case of the former. During the incubation in the presence of toluene (Exp. 3) there was no development of mould on any of the cakes, nor was there any noticeable change in appearance or smell.

As in the case of the cakes stored at the farm, the oil extracted after incubation shows a much higher free fatty acid content than before, the rise in most cases being of the same order as that found in the farm tests. The one exception is the soya cake where the rise is much greater as a result of incubation in the moist state than from storing at the farm, this difference being due probably to the fat-splitting action of the moulds formed on the cake during incubation. In contrast with

the results obtained in the farm tests a marked decrease in the percentage of oil in the dry matter was found with all the cakes, except coconut cake. There is a marked rise in the percentage of "crude protein" in the dry matter of the "soycot" and soya cakes and to a lesser extent in the cottonseed and ground-nut cakes. .During incubation in the presence of toluene (Exp. 3) there is no apparent change

TABLE III. *Incubation of Moist Cakes at 37°.*

Percentages calculated on the dry matter

Kind of cake	Before or after incubation	Crude protein	True protein	Oil	Free fatty acids as % of oleic acid in the oil
		%	%	%	%
Linseed	Before	32·78	28·77	13·43	11·23
	After (1)	33·11	30·59	9·33	56·90
	After (2)	32·83	28·88	11·18	70·50
	After (3)	32·53	28·30	13·40	36·50
Ground-nut	Before	34·08	31·21	11·27	14·65
	After (1)	37·35	34·85	4·27	71·40
	After (2)	35·95	34·23	3·93	68·60
	After (3)	32·44	31·06	10·84	62·50
Coconut	Before	20·68	19·92	15·59	5·84
	After (1)	21·25	19 78	14·72	64·10
	After (2)	20·89	19·55	15·84	61·20
	After (3)	20 37	20·08	15·65	75·40
Palm kernel	Before	19 49	18·88	10·38	5·70
	After (1)	20 13	19 51	2·95	58·90
	After (2)	19 54	19 22	4·44	64·30
	After (3)	19·19	18·87	9·99	65·70
Undecorticated cottonseed	Before	25·55	23·69	5·33	61·48
	After (1)	27·59	25·95	1·07	68 70
	After (2)	28·16	25 89	0·58	71·40
	After (3)	25·00	23·58	5·15	93·80
"Soycot"	Before	34·66	33 87	6·01	20·95
	After (1)	43·54	40·68	0·65	54·90
	After (2)	43·73	40·35	0·60	57·90
	After (3)	34·44	33·23	5·94	65·80
Soya	Before	47·94	46·35	6·61	5 18
	After (1)	55·44	52·78	4·75	56·10
	After (2)	55·96	55·14	1·54	64·00
	After (3)	47·75	46·40	6·70	9·29

The figures in brackets in the second column indicate the conditions of incubation as set out above.

in the percentage composition of the dry matter of any of the cakes examined. There is in all cases a rise in the free fatty acid content of the oil, due to the lipases present in the cakes. A clearer idea of the real significance of the changes in composition indicated can be obtained by comparing the absolute weights of each ingredient of the dry matter

present before and after incubation. For the samples used in experiments (2) and (3) it was possible, from the data obtained, to calculate these absolute weights and they are given in Table IV.

TABLE IV. *Incubation of Moist Cakes at* 37°.

Kind of cake	Before or after incubation	Total dry matter	Crude protein	True protein	Oil
		grams	grams	grams	grams
Linseed ...	Before	177·64	58·2	51·1	23·9
	After (2)	176·09	57·8	50·8	19·7
	After (3)	178 48	58·1	50·5	23·9
Ground-nut	Before	182·2	62·1	56·9	20·5
	After (2)	171·1	61·6	58 1	6·7
	After (3)	187·5	61·6	57·6	20·3
Coconut ...	Before	179 4	37·1	35·7	27·9
	After (2)	176 5	36 7	34·4	27·8
	After (3)	179·6	36 6	36·0	28·1
Palm kernel	Before	178 9	34·9	33·8	18·6
	After (2)	175·0	34·2	33·7	7·8
	After (3)	180 9	34 7	34·1	18 1
Undecorticated cottonseed	Before	176·1	45 0	41·7	9·4
	After (2)	156 7	44·1	40·6	0·9
	After (3)	177 7	44·4	41 9	9·2
"Soycot" ...	Before	176·0	61·0	59·6	10·6
	After (2)	151·0	66·0	60·9	0·9
	After (3)	180 1	62 0	59·8	10·7
Soya ..	Before	175 0	83·9	81·1	11·6
	After (2)	154 1	86 2	84·9	2·4
	After (3)	177·1	84·5	82·2	11·8

It will be seen from these figures that, during the incubation in the presence of toluene (Exp. 3) there was no loss of dry matter, crude protein or oil in any case. Under the conditions of experiment (2) an increase in weight of crude protein is shown by the "soycot" cake to the extent of 5 grams on 61 grams and by the soya cake of about half that amount. The results for these two cakes are quite abnormal in this respect as compared with the other cakes examined. On this account, further samples of cottonseed, "soycot" and soya cakes were obtained from the crushers and 100 gram lots were incubated under the conditions set out in Table V.

In this series there was no development of mould on any of the cakes until the moisture content at the commencement of the incubation exceeded 13 %, that is in the sets where the moisture content was made up to 18 % and 21 % respectively. The moulds which developed on the various cakes were examined by Miss K. Sampson, B.Sc. and in all cases species of *Aspergillus* were found, of which the following were

identified, viz.:—*A. candidus, A. herbariorum,* and *A. niger.* In addition *Mucor racemosus* and *Rhizopus nigricans* were found on the "soycot" and cottonseed cakes.

TABLE V. *Incubation of Cakes with varying Moisture Content.*

	Before incubation grams	As per sample grams	Made to 13 % grams	Made to 18 % grams	Made to 21 % grams
Undecort. Cottonseed Cake.					
Total dry matter	89·01	88·24	88·32	84·99	· 75·55
Oil	4·97	5·10	4·80	2·51	0·42
Crude protein	22·56	22·79	22·25	22·62	21·24
Crude fibre...	23·10	22·37	22·39	22·39	25·37
Sol. carbohydrates ...	33·18	32·92	33·10	32·46	23·50
Free fatty acids as % of oleic acid in oil	31·75	71·09	81·53	75·76	50·62
"*Soycot" Cake*					
Total dry matter	87·19	87·13	86·86	85·19	80·17
Oil	4·95	4·86	4·91	3 50	2·44
Crude protein	30·37	30·40	29·95	30·46	29·91
Crude fibre...	12·45	12·52	12·55	12·83	14·24
Sol. carbohydrates ...	33·61	33·40	33·52	32·44	27·74
Free fatty acids as % of oleic acid in oil	9·04	17·50	19·26	48·59	49·92
Soya Cake.					
Total dry matter	89·72	89·00	89·23	77 78	80·68
Oil	5·57	5·62	5·26	1·15	2·21
Crude protein	42·75	41·84	42 49	41 87	42·97
Crude fibre...	5·46	5·76	5·56	5·76	5·93
Sol. carbohydrates ...	30 58	30 44	30·60	23·94	24 33
Free fatty acids as % of oleic acid in oil	7·86	14 03	14·06	47·94	34·75

The foregoing results obtained with these fresh cakes and the examination of further samples of similar cakes did not confirm the previous results as to the increase in the crude protein content of "soycot" and soya cakes during incubation, and it must be concluded, therefore, that the increases shown in the first case are due to some peculiarity in the particular samples of cake used.

From an examination of the behaviour of cottonseed meal on inubation with varying water content, König(5) and Bremer(1) have shown that mould formation first occurred with a water content of 14 %, the moulds predominating until the water content exceeded 30 %, when the bacteria obtained the upper hand, the mould flora

changing with varying water content. They further found that the mould formation was always accompanied by a loss of organic matter, which in the first stages of moulding fell upon the oil fraction, but with a higher water content and the appearance of *Penicillium glaucum*, the N-free extractives were largely consumed. The results given in Tables IV and V are in full accord with these statements. In the first series (Table IV) a marked loss in dry matter only occurred where there was a development of moulds, namely in the case of the cottonseed, "soycot," soya and ground-nut cakes. In the second series (Table V) a loss in dry matter is shown only by the two sets of cakes, where the moisture content had been brought up to 18 % and 21 % respectively, and it was only on these sets that there was any mould formation. This loss in dry matter was divided between the oil and the soluble carbohydrates, falling largely on the oil fraction where the moisture content was only 18 %.

In order to determine the part played by the oil in the changes which the cakes undergo during incubation, portions of the ground cakes were extracted with light petroleum to remove the oil, the residues being dried in a steam oven and then freely exposed to the air for several hours. Portions of 50 grams of each extracted cake were then incubated for three weeks at 37' C., 8 % of water being added to the cakes, which had already taken up from 6 —10 % of moisture from the air.

TABLE VI. *Cakes extracted with Light Petroleum.*

	Before incubation			After incubation		
Kind of cake	Total dry matter	Crude protein	True protein	Total dry matter	Crude protein	True protein
	grams	grams	grams	grams	grams	grams
Linseed	45 20	16 59	14 10	44 70	16 53	13 83
Ground nut	47 80	17·22	15 83	48 00	16 86	16 03
Coconut	44 70	10 50	10 03	43 80	10 20	9 64
Palm kernel	45 19	9 37	9 26	45 10	9 18	9 11
Undecort cottonseed ...	46 67	11 76	11·65	46 20	12 25	11 80
"Soycot"	47 06	16 65	15 81	47·06	17·00	15 91
Soya	46 95	23·95	23·28	46 37	24·37	23 17

In no case was there any development of mould on the cake or any change in smell noticeable at the end of three weeks, although the cakes had been freely exposed to the air and were incubated under conditions suitable for their re-infection, owing to the presence in the incubator of other samples of cake covered with mould. As will be seen from Table VI there was no change in total dry matter or protein during the incubation. The presence of oil appears, therefore, to be one of the

conditions favouring the development of moulds on oil-cakes, and the consequent loss of organic matter.

CONCLUSIONS.

1. So far as keeping properties are concerned, palm kernel cake compares favourably with most of the oil-cakes commonly used on the farm.

2. The only change which occurs during storage under ordinary farm conditions is one which is common to all the oil-cakes examined, viz. an increase in the free fatty acid content of the oil.

3. During incubation at 37°, in a moist state, on only four of the cakes examined, namely cottonseed, ground-nut, "soycot" and soya cakes, was there any marked development of moulds.

4. This development of moulds is always accompanied by loss of organic matter, the loss being distributed between the oil and the soluble carbohydrates of the cake.

5. Moulds did not develop, during incubation, on cakes from which the oil had been previously extracted.

6. To prevent moulding of cakes and the consequent loss of organic matter, dry storage is essential. Where cakes are stored under very damp conditions serious reduction in their oil-content may take place.

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THE COMPARATIVE DIGESTIBILITY OF PALM KERNEL CAKE, EXTRACTED PALM KERNEL MEAL AND UNDECORTICATED COTTONSEED CAKE

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THE interest aroused recently in the utilisation as feeding-stuffs of the residual cake or meal remaining from the extraction of oil from palm kernels has directed attention to the scantiness of our information as to the digestibility of these products. The average digestion-coefficients given in the commonly-used tables of Kellner are based in each case upon only three experiments with two different consignments of material, and showing in certain particulars a very wide range of variation in the individual results. With one exception, where an ox was used, these experiments were carried out with sheep and all date from the 'seventies of last century[1, 2].

This information has since been supplemented by the results of trials made with sheep by Weiniger[3] in 1908, these trials including two "makes" each of the cake and extracted meal respectively.

In view of the improvements in the methods of obtaining the palm kernel oil and the consequent changes in the general character of the cake and meal it is doubtful whether the results of the older experiments can be regarded as fairly applicable to the products manufactured at the present day. Moreover in view of the fact that the production of palm kernel oil, cake and meal is largely a new industry in this country, it seemed to us desirable that information should be obtained directly as to the digestibility of the British-made products. The experiments summarised below were accordingly planned and carried out in the earlier half of 1916 at The Manor Farm, Garforth (Experimental Farm of the University of Leeds and the Yorkshire Council for Agricultural Education).

The experiments were carried out in a building specially designed for nutrition investigations and the general arrangements proved in every way adequate and satisfactory.

Two castrated male sheep, each about twelve months old and weighing 98 lbs. at the outset, were employed, No. 1 being a Wensleydale-Lincoln cross and No. 2 a Wensleydale-North Country cross. The sheep were confined during the experimental periods in wooden crates provided with feed-boxes and water-supplies. To ensure the comfort of the animals the wool was kept fairly short. Quantitative collection of the faeces was effected by means of a bag attached to the hind-quarters of the animal and held in position by means of appropriate harness. Collection of the urine was similarly effected by means of a rubber-lined leather funnel strapped to the body and communicating with a bottle placed under the crate. The general arrangements are indicated in Figs. 1 and 2.

The food-supplies were carefully weighed for each meal, the quantities being so regulated that complete consumption was effected. Composite samples of each food were made up for each period, portions being reserved daily for the purpose.

The fresh faeces after weighing were passed through a sausage mill, mixed thoroughly, and determinations of moisture and nitrogen then made without delay.

The percentage of moisture in the fresh faeces was determined in duplicate daily with large samples, by drying at 65- 70° C. The dry matter was then ground, bottled and reserved for the further analysis For this latter purpose the dried faeces were first exposed to the air in thin layers for several days, after which determinations of moisture. true protein(t), ether extract, crude fibre, ash and sand were made.

During Period I the dried faeces of each day were dealt with separately in this way, but for subsequent periods a composite sample for the period was made up, trials with the daily faeces samples of Period I having indicated that a satisfactory concordance with the mean of the records for the individual days could be obtained in this way.

The determinations of nitrogen in the fresh faeces were made daily in quadruplicate. The results thus obtained are used throughout the tables. Comparison with the total nitrogen of the dried faeces indicated a loss in drying and storage ranging from 3·1 to 6·2 per cent. (mean, 4·0 per cent.) of the total nitrogen.

The sheep were supplied with water *ad lib.* and a record kept of the actual consumption.

Fig. 1

Fig 2

Previous experience with palm kernel cake indicated that there might be some difficulty in inducing the sheep to eat it satisfactorily, and therefore for two or three weeks prior to the experiment proper the two animals were fed on a ration of "seeds" hay and palm kernel cake, the amount of each consumed during the last nine days preceding Period I being adjusted accurately to the allowance decided upon for this period. In all subsequent changes of feeding a transitional period of nine days was allowed, the change of diet being effected in the first two or three days of this interval. Each experimental period consisted of twelve consecutive days.

In order to obtain a direct comparison with some food of similar composition widely used in farm practice a period was included in which the digestibility of a sample of undecorticated cottonseed cake made from Egyptian cottonseed was included.

The general arrangement of the experiment is shown in the following schedule:

Period No.	Date from and to	Average daily ration	Starch equivalent	Nutritive ratio
I	March 14 – 25	447 gms. "Seeds" Hay + 115 gms. Palm Kernel Cake No. 1	354 gms.	1 : 7·0
II	April 4—15	447 gms. "Seeds" Hay + 115 gms. Palm Kernel Cake No. 1 + 230 gms. Palm Kernel Cake No. 2	522 „	: 5·9
III	April 25—May 6	447 gms. "Seeds" Hay + 115 gms. Palm Kernel Cake No. 1 + 230 gms. Cottonseed Cake	460 „	: 5·0
IV	May 16—27	934 gms. "Seeds" Hay	485 „	: 8·3
V	June 6—17*	446 gms. "Seeds" Hay + 345 gms. extract Palm Kernel Meal	496 „	: 5·2

* Sheep No. 2 could not be used in this period owing to unsatisfactory food-consumption, but later (July 18—28) a satisfactory period of 11 days was obtained.

In an additional period the digestibility of a sample of dried yeast was determined, but this will be dealt with in a separate communication.

The average composition of the foodstuffs is given in Table I, and of the composite samples of faeces for each period in Table II.

Before proceeding to discuss the results in detail a word must be said with regard to certain sources of error.

In the determination of digestibility by experiments of this character it is assumed that the faeces consist simply and solely of undigested residues from the food. In actual fact, however, these residues are always accompanied by appreciable quantities of metabolic products, such as bile residues, mucus and epithelial waste, so that the recorded

TABLE I. *Average Composition of Foodstuffs.*

	"Seeds" Hay %	Palm Kernel Cake I %	Palm Kernel Cake II %	Extracted Palm Kernel Meal %	Undec. Cottonseed Cake %
Moisture	9·57	10·83	11·74	11·57	11·61
Crude Protein	10·09	18·17	17·39	18·78	22·25
True Protein	8·81	17·78	17·12	18·19	20·10
Ether Extract	1·71	8·39	8·96	2·06	4·66
Nitrogen-free Extractives	49·98	44·41	44·62	47·31	33·38
Crude Fibre ... · ...	23·32	14·45	13·76	16·43	22·75
Ash*	5·33	3·75	3·53	3·85	5·35
* Including Sand ...	0·42	0 59	0·37	0·58	0 27

TABLE II. *Average Weight and Composition of Faeces.*

SHEEP 1

	Period I	Period II	Period III	Period IV	Period V
Weight of fresh faeces daily, gms.	381·4	579·5	537·7	822·0	506·5
Weight of dry matter daily, gms.	164·0	233·2	247·5	308·5	231·0

Composition of Dry Matter.

	%	%	%	%	%
Crude Protein*	14·07	14 91	16·29	12 14	14·48
Ether Extract	2·23	1·73	1·45	2·47	1·77
Nitrogen-free Extractives .	41·58	39·63	38 82	43·23	39·02
Crude Fibre	32·20	33·97	33·82	33·22	35 71
Ash†	9·92	9·76	9·62	8·94	9·02
*Including True Protein ...	12·63	13·45	14·68	10·85	12·98
*Including Pepsin-insol. Protein	7·05	7·04	9·63	6·38	7·06
†Including Sand	2·17	1·87	1·98	2 24	2·07

SHEEP 2

Weight of fresh faeces daily gms.	431·3	548·6	600·1	797·9	471·6
Weight of dry matter daily, gms.	155·7	210 5	244·4	268·9	194 2

Composition of Dry Matter.

	%	%	%	%	%
Crude Protein*	12·82	13·31	14·83	12·71	14 31
Ether Extract	2·58	1·63	1·39	2·69	1·81
Nitrogen-free Extractives ...	41·32	39·95	38·42	43·46	38 64
Crude Fibre	33·62	36·23	37·54	31·55	35·78
Ash†	9·66	8·88	7·82 ·	9·59	9·46
*Including True Protein ...	11·11	12·05	13·44	11·37	12·87
*Including Pepsin-insol. Protein	6·33	6·80	9·60	7·23	7·87
†Including Sand	2·42	2·53	2·05	2·49	3·12

digestion coefficients must inevitably be affected with error tending to give low results. The composition of these faecal products of metabolic origin is such that the error falls with special weight upon the protein and "ether extract." Repeated attempts have been made to devise a satisfactory method of discriminating between the metabolic products and the undigested food residues in the faeces with the result that, although it remains impossible to make any reliable correction of the "ether extract" figures, it is possible to make the necessary correction in the case of the protein, with apparently a fair degree of accuracy. This point is dealt with more fully in a later section of the paper and for the present it will suffice to indicate that according to the investigations of Stutzer, Kuhn, Kellner and others the digestibility of the protein of foods can be more accurately measured either by direct treatment of the foodstuff *in vitro* with an acid (HCl) solution of pepsin, or by subjecting the faeces obtained in the animal experiment to similar treatment. In the latter case it is assumed that the pepsin brings into solution the "metabolic protein," leaving the undigested food-protein intact. Recent investigations by Morgen[5] at Hohenheim indicate that the pepsin treatment alone removes only about two-thirds of the "metabolic protein" and that complete removal could only be effected by a supplementary digestion of the residue with trypsin. Until further confirmation of these results is obtained we prefer to retain the simple pepsin digestion as our basis in correction of the protein data, and the results so obtained are included in our tables.

German investigations have revealed a fairly close proportionality between the amount of organic matter digested and the amount of "metabolic protein," as measured by pepsin solubility, in the faeces Thus Kellner[6] found 0·3 to 0·5 gm. (mean, 0·4 gm.) of pepsin-soluble nitrogen to be present in the faeces per 100 gms. organic matter digested. Pfeiffer[7] obtained a similar average in experiments with pigs. but a higher figure with sheep, viz , 0·37 0·76 gm. N (mean, 0·51). Later investigations have given variable results, e.g. Katayama[8] found 0·6 0·78 gm , and Morgen[9] an average of 0·57 gm. The latter found the proportion to vary with the nature of the food, increasing to 0·82 with coarse fodder and even to 1·20 in the case of special rations containing aqueous extracts of plants. In his most recent experiments[5] he obtained an average of 0·64 gm. pepsin-soluble faecal nitrogen per 100 gms. digested organic matter, and adding one-third to this for "metabolic protein" not dissolved by pepsin arrives at the conclusion that the most probable correction factor for the digestion of

protein by sheep is 0·85 gm. nitrogen per 100 gms. digested organic matter.

In our experiments the proportion of pepsin-soluble faecal nitrogen has ranged in the different periods from 0·40 gm. to 0·63 gm. per 100 gms. of organic matter digested, with an average for the 12 comparisons of 0·52 gm. No definite correlation with the nature of the ration is traceable, the average factors for the two sheep ranging in the different periods from 0·49 to 0·54--a remarkably narrow range

As a matter of interest we have determined not only the pepsin-insoluble protein in the faeces for each period, but also the digestibility of the protein in each feeding-stuff by direct treatment *in vitro* with HCl-pepsin solution on the lines of the conventional Stutzer-Kuhn method. The results are given in the tables but may be summarised here for convenience of comparison.

Digestibility of Crude Protein

Foodstuff	as determined by animal experiment, with correction for "metabolic protein" in faeces	as determined by digestion with HCl pepsin *in vitro*
	%	%
Hay	79 2	79 7
Palm Kernel Cake I	93 6	85 7
Palm Kernel Cake II	88 5	85 2
Extracted Palm Kernel Meal .	90 0	78 3
Undecorticated Cottonseed Cake	74·7	79 7

In three cases the agreement is reasonably satisfactory, whilst in one of the other two cases (palm kernel cake I) we have reason to think, as will be explained later, that the results deduced from the animal experiment are less reliable than in the case of the other foodstuffs. The only glaring discrepancy (palm kernel meal) is not easily accounted for. The *in vitro* result given was confirmed repeatedly, and was increased only to 81·2 per cent by very prolonged (8 days) digestion of the finely-ground meal with HCl-pepsin.

Before leaving this subject attention may be directed to the relatively large amount of the faeces protein which is dissolved by the HCl-pepsin solution, fully one-half of the total nitrogenous matter being commonly removed thereby. The corrections are consequently large and there can be no doubt that the uncorrected digestion-coefficients for protein commonly used for farm feeding stuffs are in most cases seriously low.

DIGESTIBILITY OF BASAL RATION.

(*Period I.*)

We may now turn to consider the results obtained in the different periods of the experiment, taking first Period I, in which the basal ration of hay and palm kernel cake J was fed. The essential data and the digestion-coefficients deduced therefrom are summarised in Table III.

TABLE III. *Period I.* (*Basal Ration.*)

Average Daily Ration: 447·6 gms. "Seeds" Hay + 115 gms. Palm Kernel Cake I.

SHEEP 1.

	Total Dry Matter	Organic Matter	Ash (Sand-free)	Crude Protein	True Protein	Ether Extract	Nitrogen-free Extractives	Crude Fibre
	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.
Consumed:								
Clover Hay	404·8	380·9	22·0	45·2	39·4	7·65	223·7	104·4
P. K. Cake No. 1 ...	102·5	98·2	3·6	20·9	20·5	9·65	51·1	16·6
Total...	507·3	479·1	25·6	66·1	59·9	17·30	274·8	121·0
Voided	164·0	147·7	12·7 ·	23·1	20·7	3·66	68·2	52·8
Digested	343·3	331·4	12·9	43·0	39·2	13·64	206·6	68·2
Digestion Coefficients, %	67·7	69·2	50·4	65·1	65·4	78·9	75·2	56·4

SHEEP 2.

	Total Dry Matter	Organic Matter	Ash (Sand-free)	Crude Protein	True Protein	Ether Extract	Nitrogen-free Extractives	Crude Fibre
Consumed	507·3	479·1	25·6	66·1	59·9	17·3	274·8	121·0
Voided	155·7	140·6	11·3	20·0	17·3	4·0	64·4	52·3
Digested	351·6	338·5	14·3	46·1	42·6	13·3	210·4	68·7
Digestion Coefficients, %	69·3	70·6	55·9	69·8	71·1	76·9	76·6	56·8
Average Digestion Coeffi- cients, %	68·5	69·9	53·2	67·4	68·3	77·9	75·9	56·6

CORRECTED DIGESTIBILITY OF PROTEIN.

	Sheep 1		Sheep 2	
	Crude Protein	True Protein	Crude Protein	True Protein
Total pepsin-insol. protein voided, gms.	11·6	11·6	9·9	9·9
Digestion Coefficients, %	82·5	80·7	85·1	83·5
Average {Crude Protein			83·8 %	
{True Protein			82·1 %	

In view of the uncertainties of work with animals the agreement between the results yielded by the two sheep must be regarded as very satisfactory. The results for crude fibre show remarkably close agreement.

TABLE IV. *Period II. (Basal Ration + Palm Kernel Cake II.)*

Average Daily Ration: 447·6 gms. "Seeds" Hay + 115 gms. P. K. Cake I + 230 gms. P. K. Cake II.

SHEEP 1.

	Total Dry Matter	Organic Matter	Ash (Sand-free)	Crude Protein	True Protein	Ether Extract	Nitrogen-free Extractives	Crude Fibre
Consumed:	gms.	gms	gms.	gms.	gms.	gms.	gms.	gms.
Basal Ration ...	507·3	479·1	25·6	66·1	59·9	17·30	274·8	121·0
P. K. Cake II ...	203·0	194·9	7·3	40·0	39·4	20·62	102·6	31·7
Total...	710·3	674·0	32·9	106·1	99·3	37·92	377·4	152·7
Voided:—Total	233·2	210·4	18·4	34·8	31·4	4·03	92·4	79·2
From Basal Ration	164·0	147·7	12·7	23·1	20·7	3·66	68·2	52·8
From P. K. Cake II	69·2	62·7	5·7	11·7	10·7	0·37	24·2	26·4
Digested from P. K. Cake II	133·8	132·2	1·6	28·3	28·7	20·25	78·4	5·3
Digestion Coefficients of P. K. Cake II, % ...	65·9	67·8	21·9	70·8	72·9	98·2	76·4	16·7

SHEEP 2.

	Total Dry Matter	Organic Matter	Ash (Sand-free)	Crude Protein	True Protein	Ether Extract	Nitrogen-free Extractives	Crude Fibre
Consumed:—(as above)								
Voided:- Total	210·5	191·8	13·4	28·0	25·3	3·4	84·1	76·2
From Basal Ration	155·7	140·6	11·3	20·0	17·3	4·0	64·4	52·3
From P. K. Cake II	54·8	51·2	2·1	8·0	8·0	(−0·7)	19·7	23·9
Digested from P. K. Cake II	148·2	143·7	5·2	32·0	31·4	?	82·9	7·8
Digestion Coefficients of P. K. Cake II, % ...	73·0	73·7	71·3	80·0	79·7	?	80·8	24·6
Average Digestion Coefficients of P. K. Cake II, %	69·5	70·8	46·6	75·4	76·3	(98·2)	78·6	20·6

CORRECTED DIGESTIBILITY OF PROTEIN.

	Sheep 1		Sheep 2	
	Crude Protein	True Protein	Crude Protein	True Protein
Total pepsin-insol. protein voided, gms.	16·4	16·4	14·3	14·3
Deduct for basal ration, gms.	11·6	11·6	9·9	9·9
Pepsin-insol. protein voided from P. K. Cake II, gms.	4·8	4·8	4·4	4·4
Digestion Coefficients, %	88·0	87·8	89·0	88·8
Average { Crude Protein		88·5 %*		
{ True Protein		88·3 %		

* Coefficient obtained by direct treatment of the cake with pepsin-HCl was 85·2 %.

DIGESTIBILITY OF PALM KERNEL CAKE II.

(*Period II.*)

Passing now to the records of Period II in which palm kernel cake II was fed along with the basal ration, these are summarised in Table IV, which indicates also how by deducting the contribution of the basal ration to the faeces as ascertained in Period I, the digestibility of the added palm kernel cake is arrived at.

The concordance between the results given by the individual sheep for Period II is not quite so good as for Period I but may still be regarded as reasonably good for work of this character, especially in view of the fact that what is being measured is the relatively small difference between the two rations.

Sheep 2 again gives indications of slightly higher digestive powers than Sheep 1, but the difference is barely 4 per cent. of the organic matter digested.

In the case of Sheep 2 no definite conclusion as to the digestibility of the oil of the palm kernel cake can be drawn since the total weight of "ether extract" voided in Period II was actually less than that of Period I. The explanation of the apparent anomaly lies, as already pointed out, in the presence in the faeces of ether-soluble products of metabolic origin. It is quite evident from the records of both sheep that the palm kernel oil has contributed little or nothing to the "ether extract" of the faeces, and for all practical purposes it may be taken as entirely digestible.

DIGESTIBILITY OF UNDECORTICATED COTTONSEED CAKE.

(*Period III.*)

Table V summarises the results obtained in Period III and the deduction therefrom of the digestion-coefficients of the cottonseed cake used to supplement the basal ration in this period. Except for the difference in the nature of the cake the ration was identical with that consumed in Period II.

The concordance between the two sets of results is again very good, with the exception of the crude fibre. Such divergence as is here shown with regard to the latter is unfortunately very common in digestion

TABLE V. *Period III.* (*Basal Ration + Undecorticated Cottonseed Cake.*)

Average Daily Ration: 447·6 gms. "Seeds" Hay + 115 gms. Palm Kernel Cake I + 230 gms. Cottonseed Cake.

SHEEP 1.

	Total Dry Matter	Organic Matter	Ash (Sand-free)	Crude Protein	True Protein	Ether Extract	Nitrogen-free Extractives	Crude Fibre
Consumed:	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.
Basal Ration ...	507·3	479·1	25·6	66·1	59·9	17·30	274·8	121·0
Cottonseed Cake ...	203·3	191·0	11·7	51·2	46·2	10·72	75·3	53·8
Total...	710·6	670·1	37·3	117·3	106·1	28·02	350·1	174·8
Voided:—Total	247·5	223·6	18·9 ·	40·3	36·3	3·60	96·0	83·7
From Basal Ration ...	164·0	147·7	12·7	23·1	20·7	3·66	68·2	52·8
From Cottonseed Cake	83·5	75·9	6·2	17·2	15·6	(- 0·06)	27·8	30·9
Digested from Cottonseed Cake	119·8	115·1	5·5	34·0	30·6	?	47·5	22·9
Digestion Coefficients of Cottonseed Cake, % ...	59·0	60·3	47·0	66·4	66·2	?	63·1	42·5

SHEEP 2.

	Total Dry Matter	Organic Matter	Ash (Sand-free)	Crude Protein	True Protein	Ether Extract	Nitrogen-free Extractives	Crude Fibre
Consumed:—(as above)								
Voided: - Total	244·4	225·3	14·1	36·2	32·9	3·4	93·9	91·8
From Basal Ration ...	155·7	140·6	11·3	20·0	17·3	4·0	64·4	52·3
From Cottonseed Cake	88·7	84·7	2·8	16·2	15·6	(0·6)	29·5	39·5
Digested from Cottonseed Cake	114·6	106·3	8·9	35·0	30·6	?	45·8	14·3
Digestion Coefficients of Cottonseed Cake, % ...	56·4	55·7	76·1	68·4	66·2	?	60·8	26·6
Average Digestion Coefficients of Cottonseed Cake, %	57·7	58·0	61·6	67·4	66·2	?	62·0	34·6

CORRECTED DIGESTIBILITY OF PROTEIN.

	Sheep 1		Sheep 2	
	Crude Protein	True Protein	Crude Protein	True Protein
Total pepsin-insol. protein voided, gms.	23·9	23·9	23·5	23·5
Deduct for basal ration, gms.	11·6	11·6	9·9	9·9
Pepsin-insol. protein voided from Cottonseed Cake, gms.	12·3	12·3	13·6	13·6
Digestion Coefficients, %	76·0	73·4	73·4	70·6
Average { Crude Protein		74·7 %*		
{ True Protein		72·0 %		

* Coefficient determined by direct treatment of the cake with pepsin-HCl was 79·7 %.

trials. The average results agree fairly well with the following averages of ten experiments as given by Kellner:

	Percentage Digestibility	
	Range of Variation	Average %
Organic Matter	50—60	55
Crude Protein	72—77	77
Ether Extract	86—100	93
Nitrogen-free Extractives ...	46—60	52
Crude Fibre	2—24	(18)

It is evident that so far as digestibility is concerned little fault could be found with the cottonseed cake used in our experiment.

DIGESTIBILITY OF "SEEDS" HAY.
(*Period IV.*)

Table VI summarises the results obtained in Period IV, during which the sheep were fed on hay alone.

TABLE VI. *Period IV.* (*"Seeds" Hay only.*)
Average Daily Ration: 934·4 gms. "Seeds" Hay.

SHEEP 1.

	Total Dry Matter · gms.	Organic Matter gms.	Ash (Sand-free) gms	Crude Protein gms	True Protein gms.	Ether Extract gms	Nitrogen-free Extractives gms.	Crude Fibre gms.
Consumed	845·0	795·2	45·9	94·9	82·3	15·98	467·0	217 9
Voided	308·5	280·9	20·7	37·5	33·5	7·63	133·4	102·5
Digested	536 5	514·3	25·2	56 8	48 8	8·35	333 6	115 4
Digestion Coefficients, %	63 5	64 7	54 9	60·3	59·3	52 2	71·4	53 0

SHEEP 2.

	Total Dry Matter · gms.	Organic Matter gms.	Ash (Sand-free) gms	Crude Protein gms	True Protein gms.	Ether Extract gms	Nitrogen-free Extractives gms.	Crude Fibre gms.
Consumed: (as above)								
Voided	268 9	243 1	19·8	34·2	30·6	7·23	116·9	84·8
Digested	576·1	552·1	26·1	60·1	51·7	8·75	350·1	133·1
Digestion Coefficients ...	68·2	69·4	56·9	63·7	62 9	54 7	75·0	61·1
Average Digestion Coefficients, %	65·8	67·0	55·9	62·0	61·1	53·5	73·2	57·0

CORRECTED DIGESTIBILITY OF PROTEIN.

	Sheep 1		Sheep 2	
	Crude Protein	True Protein	Crude Protein	True Protein
Total pepsin-insol. protein voided, gms.	19·7	19·7	19·44	19·44
Digestion Coefficients, %	79·1	76·1	79·4	76·4
Average (Crude Protein		79·2 %*		
(True Protein		76·2 %		

* Coefficient determined by direct treatment of the finely-ground hay with pepsin-HCl was 79 7 %.

The agreement between the records of the two animals is fairly good throughout, not excepting even the coefficients for ether extract and crude fibre. The average coefficients combined with the data previously given (p. 433) for the composition of the hay indicate that it was of very good quality, the digestibility of the organic matter actually exceeding that found for the organic matter of the cottonseed cake in the preceding period.

TABLE VII. *Digestibility of Palm Kernel Cake 1.*
(*Periods I and IV*).

SHEEP 1.

	Total Dry Matter	Organic Matter	Ash (Sand-free)	Crude Protein	True Protein	Ether Extract	Nitrogen-free Extractives	Crude Fibre
	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.
Consumed (Period I):								
Palm K. Cake I ...	102·5	98·2	3·6	20·9	20·5	9·65	51·1	16·6
Digested (Period I):								
Total:	343·3	331·4	12·9	43·0	39·2	13·64	206·6	68·2
Hay (calc. from Per. IV)	257·0	246·4	12·1	27·2	23·4	4·00	159·8	55·3
Palm K. Cake I ...	86·3	85·0	0·8	15·8	15·8	9·64	46·8	12·9
Digestion Coefficients of								
Palm K. Cake I, % ...	84·2	86·6	22·2	75·6	77·1	99·9	91·5	77·7

SHEEP 2.

	Total Dry Matter	Organic Matter	Ash (Sand-free)	Crude Protein	True Protein	Ether Extract	Nitrogen-free Extractives	Crude Fibre
Digested (Period I):								
Total:	351·6	338·5	14·3	46·1	42·6	13·3	210·4	68·7
Hay (calc. from Per. IV)	276·0	264·5	12·5	28·8	24·8	4·2	167·7	63·8
Palm K. Cake I ...	75·6	74·0	1·8	17·3	17·8	9·1	42·7	4·9
Digestion Coefficients of								
Palm K. Cake I, % ...	73·7	75·3	50·0	82·7	86·8	94·3	83·5	29·5
Average Digestion Coefficients of Palm Kernel Cake I, %	78·9	80·9	36·1	79·2	82·0	97·1	87·5	53·6

CORRECTED DIGESTIBILITY OF PROTEIN.

	Sheep 1		Sheep 2	
	Crude Protein	True Protein	Crude Protein	True Protein
Total pepsin-insol. protein voided, Period I, gms. ...	11·56	11·56	9·86	9·86
Pepsin-insol. protein voided from hay (calc. Period IV), gms.	9·44	9·44	9·32	9·32
Pepsin-insol. protein voided from Palm K. Cake I (Period I), gms.	2·12	2·12	0·54	0·54
Digestion Coefficients, %	89·8	89·6	97·40	97·4
Average { Crude Protein			93·6 %*	
{ True Protein			93·5 %	

* Coefficient determined by direct treatment of cake with pepsin-HCl was 85·7 %.

DIGESTIBILITY OF PALM KERNEL CAKE I.

By using now these data for the digestibility of the hay and applying them to the records for Period I (Table III) in which a mixture of hay and palm kernel cake I was fed we can deduce the digestion coefficients of the latter. The results of this estimation are summarised in Table VII.

In certain respects the agreement between the two sheep is not satisfactory, the coefficients for the organic matter differing by over 11 per cent. Comparison with the results obtained with palm kernel cake II in Period II (Table IV) suggests that the results obtained with Sheep 1 are too high, this certainly being the case with the indicated digestion coefficient for crude fibre.

These discrepancies in the results for this palm kernel cake were not unexpected since not only was the amount used small (115 gms. per head per day), but the ration containing it (Period I) was much less bulky and contained less dry matter than the hay ration of Period IV from which the digestibility of the hay was determined. There is little doubt that the disturbing factor of the presence of metabolic products in the faeces would make itself felt, since the amount of such products present is not necessarily proportional to the weight of dry matter consumed. This doubtless explains why the divergence between the two sheep is greatest in respect of the digestion coefficients for protein.

SUMMARY OF RESULTS WITH PALM KERNEL CAKE.

Leaving out of account the apparently abnormal results given by Sheep 1 for palm kernel cake I, the three remaining determinations of the digestibility of palm kernel cake have given the results summarised below:

	Palm Kernel Cake I Sheep 2	Palm Kernel Cake II		Average of all foregoing	Average (Weniger)	Average (Kellner)
		Sheep 1	Sheep 2			
	%	%	%	%	%	%
Organic Matter	75 3	67·8	73·7	72·3	76·5	70
*Crude Protein	82·7	70·8	80·0	77·8	76·6	75
„ „ (corrected)	97·4	88·0	89·0	90·2	—	—
*True Protein	86·8	72·9	79·7	79 8	—	—
„ „ (corrected)	97·4	87·8	88 8	90·0	—	—
Ether Extract	94·3	98·2	(100?)	97·5	78 7	98
Nitrogen free Extractives	83·5	76·4	80·8	80·2	88·8	77
Crude Fibre	29·5	16·7	24·5	23·6	39·4	39
* As determined *in vitro*	85·7	85·2		85·5	—	—

For purpóses of comparison the average of Weiniger's results with two brands of palm kernel cake and the general average given by Kellner are included in the table. The Garforth averages in the main are intermediate between the two German averages, crude fibre being however a marked exception. It would seem that Kellner's averages, apart possibly from crude fibre, are not far wide of the mark, being if anything slightly on the low side. His average for crude protein agrees closely with our uncorrected average, but is of course considerably below the corrected average. We incline, on the whole, to regard the results obtained *in vitro* as the most nearly correct expression of the digestibility of the protein.

DIGESTIBILITY OF EXTRACTED PALM KERNEL MEAL.

(*Period V.*)

After Period IV the original basal ration of hay and palm kernel cake was not reverted to, but the sheep received an allowance of hay reduced to the amount consumed in the earlier periods and to this was added the extracted palm kernel meal. The digestibility of the latter is thus deduced from the records of Periods IV and V, as summarised in Table VIII.

The concordance between the two sheep is only moderately good throughout. The explanation doubtless lies partly in the less bulky nature of the ration in Period V as compared with Period IV, and consequent irregularities in the incidence upon the results of the metabolic products in the faeces.

The three sets of averages for extracted palm kernel meal are given below:

	Garforth Average %	Weiniger %	Kellner %
Organic Matter	76·7	79 7	91
*Crude Protein	79·2	74 1	95
„ „ (corrected) ...	90·0	—	—
Ether Extract	96·3	?	95
Nitrogen-free Extractives ...	86·0	92·6	94
Crude Fibre	44·8	55 2	82
* As determined *in vitro* ...	78·3	—	—

If the Garforth average be compared with that for palm kernel cake given on p. 442, it will be seen that the extracted meal is indicated as being rather more digestible than the cake. Except for crude fibre, however, the differences are not great and probably little more than

There can be little doubt that Kellner's figures for organic matter, protein and fibre in the case of the meal are much too high. It is difficult, however, to resist the conclusion that the meal is in fact rather more highly digestible than the cake, and that this conclusion applies especially to the crude fibre, although it is not clear why such . should be the case.

COMPARISON OF PALM KERNEL CAKE AND MEAL WITH UNDECORTICATED COTTONSEED CAKE.

It was explained at the outset that Period III was included in this experiment in order that a direct comparison might be made between palm kernel cake and undecorticated cottonseed cake, the latter being selected as an oil-cake in wide use and not widely different in general chemical composition from palm kernel cake.

Of the two palm kernel cakes used it is desirable to restrict the comparison to palm kernel cake II, since the conditions under which its digestibility was determined (Period II) were strictly comparable with those of Period III, the weights of hay and cake consumed being exactly the same in each. For the palm kernel meal only the one pair of determinations is available.

The average digestion coefficients of the two cakes, and of the meal, are reproduced below:

	Undecorticated Cottonseed Cake	Palm Kernel Cake II	Extracted Palm Kernel Meal
	%	%	%
Organic Matter	58 0	70·8	76 7
Crude Protein	67·4	75·4	79 2
„ „ (corrected)	74·7	88·5	90·0
Ether Extract	(100?)	98·2	96 3
Nitrogen free Extractives	62·0	78·6	86 0
Crude Fibre	34·6	20 6	44·8

It is obvious at a glance that the cottonseed cake compares very unfavourably with the palm kernel cake, and hence still more so with the palm kernel meal. In view of claims for relatively high digestibility made by Kellner it may be noted however that the crude fibre of the palm kernel foods did not surpass in digestibility that of the cottonseed cake, the coefficient for the latter being practically identical with the average of the former.

Applying the foregoing coefficients to the composition of each feeding-stuff as given in Table I we arrive at the following percentages of digestible ingredients:

	Undecorticated Cottonseed Cake	Palm Kernel Cake II	Extracted Palm Kernel Meal
	%	%	%
Organic Matter	48·2	60·0	64·9
Crude Protein	16·6	15·4	16·9
Ether Extract	4·5 (est.)	8·8	2·0
Nitrogen-free Extractives	20·7	35·1	40·7
Crude Fibre	7·9	2·8	7·3
"Food Units"	81·4	98·4	95·2
Starch Equivalent (Kellner)	46·2	73·6	68·7

The "food units" included in the table are calculated by the conventional expression and are designed to furnish a measure of the relative money values of the feeding-stuffs, whilst the starch equivalents give a measure of their feeding value when added to a maintenance ration. These data indicate that the palm kernel cake was a little superior to the meal both in money value and in feeding value, whilst its superiority over the cottonseed cake was roughly 20 per cent. in money value and 60 per cent. in feeding value. These comparisons naturally have reference only to the materials actually used in the experiments.

NITROGEN BALANCE.

Although not essential for the estimation of digestibility it was thought desirable throughout the experiments to collect the urine voided by the sheep and determine the nitrogen therein. A complete survey of the utilisation of the food nitrogen was thus obtained, and is summarised in Table IX:

TABLE IX. *Nitrogen Balance.*

Period	Nature of Ration		Nitrogen Consumed, Average per day	Nitrogen Voided (Average per day) In Faeces	In Urine	Total	Nitrogen Retained (+) or lost (−) by sheep, Average per day
			gms.	gms.	gms.	gms.	gms.
I	Hay and Palm Kernel Cake I ...	Sheep 1	10 57	3 69	7 25	10·94	− 0·37
		„ 2	10·57	3 19	5·43	8·62	+ 1·94
II	Hay and P. K. Cake I and II ..	Sheep 1	16 96	5 56	7·60	13 16	+ 3 80
		„ 2	16·96	4·48	7·72	12·20	+ 4·76
III	Hay + Undec. Cottonseed Cake...	Sheep 1	18 76	6·45	9·06	15 51	+ 3·25
		„ 2	18·76	5 80	9·20	15 00	+ 3·76
IV	Hay	Sheep 1	15·08	5·99	7·17	13·16	+ 1·93
		„ 2	15·08	5·47	7·47	12 94	+ 2 14
V	Hay + Extr. Palm Kernel Meal	Sheep 1	17·57	5·35	8·89	14·24	+ 3·33
		„ 2	17·57	4·45	10·97	15·42	+ 2·15
VI	Hay + Dried Yeast	Sheep 1	22 78	4·68	15 16	19·84	+ 2·94
		„ 2	22·78	4·50	16·93	21·43	+ 1·35

It will be observed that with the exception of Sheep 1 in Period I a positive nitrogen balance was maintained throughout. An interesting correlation between the amounts of nitrogen consumed and the amounts of nitrogen retained becomes evident if the data be rearranged in order of increasing nitrogen consumption as set out in the following table:

Period	Nitrogen consumed	Nitrogen retained by Sheep	
		Sheep 1	Sheep 2
	gms	gms.	gms.
I	10·57	− 0·37	1·94
IV	15·08	1·93	2·14
II	16·96	3·80	4·76
V	17·57	3·33	2·15
III	18 76	3·25	3·76
VI	22·78	2·94	1·35

It will be seen that with the exception of Sheep 2, Period V, in each case as the nitrogen consumption was increased the nitrogen retention increased up to a certain point and then fell, the maximum retention being recorded by each sheep in Period II (Hay ¦ Palm Kernel Cakes), in which roughly 17 gms. of nitrogen (= 106 gms. crude protein) per head per day were consumed. This amount is equivalent to 2·4 kg. crude protein per 1000 kg. live-weight, the nutritive ratio of the ration being practically 1 : 6. The estimated "starch equivalent" of the ration moreover was highest in this period (cf. p. 132).

The lower retention in Periods I and IV is doubtless due to the smaller consumption of nitrogenous matter, but it is not clear why less nitrogen should have been retained in Periods III, V and VI. It is hoped that further studies now in progress will throw light on this point.

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It will be noticed that with neither sheep was it possible to measure the digestibility of the ether extract and crude fibre of the dried yeast owing to the very small proportions in which these ingredients were present. For the rest the agreement between the two sheep is eminently satisfactory and the average indicates a very high order of digestibility, despite the apparently "low grade" quality of the sample.

The results compare favourably with those obtained by Honcamp[1] using material containing 56·1 % of crude protein. This material gave digestion coefficients as follows: organic matter 81·5 %; crude protein 86·6 %; nitrogen-free extractives 81·5 % and ether extract 38·2 %.

The low figure for the ether extract is perhaps doubtful, but in any case is of little significance since dried yeast is extremely poor in oil.

Recent measurements[2] of the digestibility of dried yeast by the human subject indicate a very low order of digestibility, but it is clear from the results given above that this is not the case with sheep, and therefore presumably with cattle. On the contrary dried yeast must rank with the most highly digestible foods used on the farm.

[1] F. Honcamp, et al. *Landwirts. Versuchsstat.* **73**, p. 271.

[2] C. Funk, et al. *Journ. Biol. Chem.* **27** (1916), 173

THE INFLUENCE OF PALM KERNEL CAKE UPON THE COMPOSITION OF MILK-FAT.

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THE experiment described in the present communication was carried out at the Manor Farm, Garforth, during the summer of 1916, with the object of obtaining information as to the nature of any changes in the composition of milk-fat that might be effected by the feeding of palm kernel cake to cows.

Two cows each yielding 2 to 3 gallons of milk per day were selected for the purpose in May, and for two or three weeks prior to the commencement of the experiment were out at pasture day and night without any supplementary allowance of cake or other food. The experiment was divided into three periods of three weeks each, with two transitional periods of one week each. The way in which these periods were utilised will be clear from the appended schedule:

Period	Nature of Feeding
I. (June 4—24)	Grass alone
1st transitional (June 25—July 1) ...	Grass + 2 lb. Palm Kernel Cake
II. (July 2—22)	,, ,, ,, ,, ,,
2nd transitional (July 23—29) ...	Grass alone
III. (July 30—Aug 19)	,,

It will be seen that the pasturage without cake was continued for the first three weeks, then the supplementary cake allowance introduced and continued for four weeks, after which simple pasturage was reverted to for the remaining four weeks. The feeding in Periods I and III thus being the same, a comparison of the records for these periods indicates the direction and extent of changes due to the combined effects of advance of lactation and variation in quality of pasture. In each of these periods of four weeks, the first week is treated as transitional.

It was intended to give a higher allowance of cake, but the cows, presumably on account of the abundant pasturage, could not be induced to eat regularly more than 2 lb. per day.

In each week, with the exception of the first week of Period I, samples

Cow No. 23.

Date of Sampling	Koettstorfer Number	Reichert-Wollny Number	Polenske Number	Iodine Absorption Number	Refractive Index at 40° C.
1916	mg. KOH	c.c. N/10 NaOH	c.c. N/10 NaOH	gms. Iodine	
Period I June 7, 8	228·5	30·2	2·4	43·6	1·4529
,, 12, 13	225·3	30·6	2·7	41·0	24
,, 14, 15	227·5	32·5	2·7	42·6	25
,, 19, 20	229·6	30·2	2·5	41·1	22
,, 21, 22	231·0	31·3	2·6	40·1	16
Transl. week June 26, 27	229·4	29·5	2·0	39·6	1·4520
,, 28, 29	231·0	31·1	2·1	39·1	15
Period II July 3, 4	230·5	31·3	3·1	38·4	1·4514
,, 5, 6	230·6	30·5	2·5	37·2	13
,, 10, 11	231·4	30·2	2·4	37·4	14
,, 12, 13	229·8	29·6	2·4	39·1	14
,, 17, 18	227·6	28·7	2·3	37·0	15
,, 19, 20	230·4	29·6	2·1	36·5	12
Transl. week July 24, 25	227·9	27·6	2·0	38·0	1·4517
,, 26, 27	230·1	25·4	1·6	39·6	22
Period III July 31, Aug. 1	228·2	27·3	1·5	41·5	1·4528
Aug. 2, 3	226·7	26·7	1·3	42·4	30
,, 7, 8	226·0	25·0	1·2	41·7	28
,, 9, 10	218·6	26·0	1·1	42·5	30
,, 14, 15	217·0	24·4	1·2	42·2	29
,, 16, 17	218·6	24·2	1·2	45·6	30

Summary.

	Koettstorfer Number	Reichert-Wollny Number	Polenske Number	Iodine Absorption Number	Refractive Index at 40° C.
(a) Average, Period I	228·4	31·0	2·55	41·7	1·4523
(b) ,, Period II	230·1	30·0	2·47	37·6	1·4513
(c) ,, Period III	222·5	25·6	1·24	42·7	1·4529
(d) Mean of Periods I and III	225·4	28·3	1·90	42·2	1·4526
Effect of Palm Kernel Cake (b—d)	+4·7 ± 1·0	+1·7 ± ·6	+0·57 ± ·2	−4·6 ± ·4	−0·0013 ± ·0001

These are precisely the effects recorded in the present experiment, with the exception of the apparent slight raising of the Reichert-Wollny number. If the differences recorded with regard to this criterion may be taken as due in fact to the palm kernel feeding we can only conclude that the palm kernel oil has not passed as a whole into the milk-fat but that its component acids have been subjected to a selective action, whereby the acids of relatively low molecular weight have been transferred to the milk in greater proportion than the higher acids. The differences are too slight, however, to warrant any definite conclusion of this character.

(Received February 22nd, 1917.)

ON FORMS OF THE HOP (*HUMULUS LUPULUS* L.) RESISTANT TO MILDEW (*SPHAEROTHECA HUMULI* (DC.) BURR.).

By E. S. SALMON.

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In certain seasons very severe losses are caused to the hóp-crop by the depredations of the mildew *Sphaerotheca Humuli* (DC.) Burr. Among hop-growers in England this mildew is commonly spoken of as "mould" when it òccurs on the leaves or the female inflorescences ("burr") of the hop-plant, and as "red mould" when it attacks the stipular bracts and bracteoles of the strobiles ("hops")[1][1]:

The other serious pest of hop-growing, viz., the "blight" (*Phorodon Humuli* Schrank), is satisfactorily held in check by modern methods of washing with an insecticide, but I have frequently been assured by experienced hop-growers that while they feel competent to deal with even prolonged attacks of "blight," they view with great uneasiness the appearance of "mould" in the hop-garden, especially in dull, damp seasons when sulphuring appears to be of comparatively little use.

During the past season (1916) the ravages of "red mould" in the hop-gardens in all the hop-growing centres of this country resulted in losses of many thousands of pounds, since some hundreds of acres had to be left unpicked owing to the hops becoming too "mouldy," while in those cases where the attack was slighter, and the hops were picked, the presence of any "mouldy" hops (strobiles) when detected in the sample invariably meant for the grower a serious lowering of their market price.

Within recent years the hop-mildew has appeared in epidemic form in the "hop-yards" in the United States [2, 3].

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[1] For reference to these numbers, see "Literature cited," at end of this article.

It is a matter, therefore, of economic as well as of scientific interest, to find that certain forms of the hop-plant, *Humulus Lupulus* L., are markedly resistant to *S. Humuli*.

It may be pointed out here that while the morphological species *S. Humuli* is found attacking a very large number of host-species belonging to many diverse genera (5, 6), it has been proved by experiments that a specialisation of parasitism has taken place and that the form on *Humulus* constitutes a "biologic form" with power of infecting only, so far as is known (4), species of the genus *Humulus*.

The phenomenon of resistance to hop-mildew was first observed during the summer of 1914. For the purpose of carrying out trials with certain fungicides, several hundreds of one- and two-year old seedling hops were grown in pots in a glass-house under conditions which ensured their being virulently attacked by the mildew (10). The conditions provided, viz. alternations of a dry and damp atmosphere and the occurrence of constant draughts, enabled the mildew to attack continuously the leaves and stems of the seedling hops to the maximum extent, and, generally speaking, it was only necessary to place a healthy hop-plant among the infected plants in this glass-house to find it at the end of a week or fortnight smothered with mildew.

Under what were clearly ideal conditions for the growth and dissemination of the hop-mildew, two individual seedlings proved immune throughout the growing season. On these plants alone among those in the glass-house there was not a spot of mildew on leaf or stem, The two plants were moved from time to time, and placed among batches of the most virulently infected plants, from which conidia must have been blown repeatedly on to their leaves and stems. Direct inoculations were also made either by placing conidia on the damped surface of a leaf, or by first spraying the plant with water (using an "atomiser") and shaking over it affected plants bearing numerous "powdery" conidial patches, so that myriads of conidia fell in a cloud on to the inoculated plant. The two plants remained persistently immune. These plants were seedlings raised from seed of the "wild hop" obtained from Italy. Other seedlings, to the number of about seventy, of the same origin proved very susceptible to the mildew. These susceptible seedlings were of the same age as the immune plants, and were raised in the glass-house under the same conditions as regards soil and all other cultural matters.

During 1916 the same phenomenon was observed in the case of seven other seedlings of the same origin. They were of the same age as the

immune plants observed in 1914, a second sowing of the seed having been made in 1915. The resistance to mildew of these seven plants was put to the same tests as in 1914, and all the plants remained immune throughout the growing season. Other seedlings (to the number of about one hundred and fifty) of the same origin and age and grown in the same glass-house proved highly susceptible to mildew.

The same complete immunity was also shown, during 1916, under the same circumstances, by a form of *Humulus Lupulus* with yellow leaves, obtained under the name of "golden hop" from Messrs Bide and Sons, of Farnham. In this case the plants, three in number, were "cuts" taken from an older plant or plants, and potted up the previous autumn. In one experiment, on May 4, 1916, two young leaves of this "golden hop," and one young leaf of a seedling hop, were removed from the plants, inoculated, and placed on damp blotting-paper in a large petri dish. By May 9 the green leaf of the seedling showed at the place of inoculation several vigorously-growing patches of mildew, with numerous clustered conidiophores; by May 15 these patches had become densely "powdery." No trace of infection resulted on the leaves of the "golden hop."

The two immune seedlings noticed in 1914 were planted out during the winter 1914–15 in the Wye College Experimental Hop-garden, and were sufficiently established by 1916 to grow to the normal length of an adult plant and to produce flowers. One plant proved to be female, the other, male. The season of 1916 was, as mentioned above, notable for the prevalence of "mould" in hops, and in the part of the hop-garden where the two seedlings were planted the surrounding "hills" of hops showed, during the summer, patches of mildew on the leaves and hops. The two seedlings showed no trace of mildew on any part through the summer and early autumn, although mildew was noticed on all the adjacent plants. In October, however, mildew was observed on both the seedlings. By October 3 one plant (the female) had produced a late, rather weak shoot ("bine") which had run up round the old stems ("bines") to a considerable height and produced a few hops. On this late shoot alone some of the leaves and one hop bore several small patches of mildew. The other plant (male) similarly remained resistant to mildew throughout the growing season; on October 3 two late lateral shoots each showed one leaf with small patches of mildew.

The further facts as regards the origin of these plants which have shown resistance to mildew are as follows.

With regard to the nine seedlings of the "wild hop" from Italy, the seed was kindly sent to me by Prof. P. A. Saccardo in 1913, labelled "Semina *H. Lupuli*, Oct. 1913. Vittorio (Treviso), ad sepes, omnino sponte. In Italia *Humulus* non colitur." Subsequently, however, I was informed, in 1915, by Dr M. Corvi, of the R. Instituto Superiore Agrario, of Perugia, that in the years 1860-70 the hop was cultivated near Bologna "with good results," and that experiments with its culture were now being conducted in Umbria; thus the certainty of hop-seed obtained from Italy being that of the truly wild *H. Lupulus* appeared to be called in question. The following information, however, supplied by Prof. P. A. Saccardo in April, 1916, makes it practically certain that the parent plant of the resistant seedlings was the wild species : — "Après investigations chez mes collègues Mittirolo, Peglion, Béguinot, &c. je peux vous assurer,—*L'houblon* n'a été *jamais* cultivé dans la province de Treviso d'où je vous l'ai expédié et où il se trouve *sauvage partout abondamment*, surtout sur les haies. M. le prof. Peglion qui va publier dans ce moment un *Monografia del luppolo in Italia* m'écrit de Bologna que en toute Italie on cultive : 3 *hectares* de houblon dans la plaine d'Orvieto et dans l'haute-plaine de Alfina (Ombria), ½ *hectare* à Pedavena (Feltre). Comme vous le voyez vous pouvez être sûr, très sûr, que les exemples que je vous ai expédiés sont absolument *spontanés* et *sauvages*."

Some of the seedlings of the same Italian parentage which have been planted out in the hop-garden have proved to be extremely susceptible to the mildew, far more so than any of the numerous cultivated varieties planted round them.

It is clear, then, that the species *H. Lupulus* in its wild state comprises forms which may be either very susceptible or very resistant to the attacks of *S. Humuli*. Whether these forms are identical morphologically or not, cannot be determined until a detailed comparison of the mature plants becomes possible.

With regard to the form with yellow leaves, Messrs Bide inform me that they bought the plant from M. G. Benard, of Orleans, France. I have not been able to obtain the history of its origin.

Neger, in 1902, pointed out the possibility of the existence of "immune races" among mildew-susceptible host-species, and described(11) instances where individual plants of *Spiraea Ulmaria* and *Epilobium montanum* resisted all infection from the conidia of the *Sphaerotheca* (*S. Humuli*) on the same host-species respectively. In another case a plant of *Ranunculus repens* proved immune under

repeated inoculations with conidia of *Erysiphe Polygoni* growing on another individual of *R. repens*[1].

From the observations of Biffen[12], Reed[13], Wawilow[14] and the writer[9], it is clear that with respect to another species of mildew, viz. *Erysiphe Graminis*, there occur, among the host species of its "biologic forms," varieties or forms which are either immune or strongly resistant.

According to the following communication from Prof. Kingo Miyabe, the "biologic form" of *S. Humuli* which attacks *Humulus* does not occur in Japan, although the species *S. Humuli* is common in that country on a number of host-species belonging to different genera[7,8]. Prof. Kingo Miyabe writes: – "One thing which strikes me as most peculiar is the entire absence of the powdery mildew on both the wild and cultivated hop-plants in the vicinity of Sapporo and also probably in the rest of our country. The cultivated hop has been introduced from Europe, mostly from Germany." (It may be noted here, however, that Hennings has recorded[15] *S. Humuli* from Japan: "Tokyo: auf Blättern von *Humulus Lupulus* L. (Shirai)[2].")

In 1916 I received from Prof. Kingo Miyabe examples of the male plant of the wild hop of Japan (*H. Lupulus* var. *cordifolius* Maxim); on growing them in the glass-house where, as mentioned above, all the conditions were favourable to inoculation by conidia of the hop-form of *S. Humuli*, the plants became infected.

A cognate case is doubtless that of the "immunity" of the "Virginia Creeper" (*Vitis hederacea*) in Europe. In the United States this plant is attacked by the mildew *Uncinula necator* (Schwein.) Burr. This mildew is very common in Europe on cultivated vines, but so far as I know has never been found attacking the "Virginia Creeper," doubtless because specialisation of parasitism has occurred in the species *U.*

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[1] In 1907 Dr W. W. Stockberger, of the United States Department of Agriculture, recorded ("Improving the Quality of Domestic Hops"; paper read at a Meeting of the American Brewing Institute, Nov. 20: 1907) the following fact: "During the past season in some of the badly moulded yards of New York State, hills were found which were absolutely free from mould, although entirely surrounded by badly moulded hills. These hills have been marked, and cuttings will be taken from them next spring and used for propagating these resistant individuals." In a recent communication to me Dr Stockberger informs me that the proposed plan of testing these varieties was never carried out; further, he now states that in his opinion too much importance as evidence of immunity must not be attached to the phenomenon recorded by him in 1907.

[2] The second record of *S. Humuli* on *Humulus* in Japan given in my "*Erysiphaceae* of Japan," II. *Annal Mycolog*, III, p. 252 (8), is an error of transliteration, the host-species being *Impatiens Balsamina* L., Tokyo Botanic Garden, Coll. Kusano.

necator, and the "biologic form" attacking *Vitis hederacea* has not yet reached Europe[1].

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[1] At the present time no restrictions exist as to the importation from America of *Vitis hederacea.* If our official Horticultural Authorities wish to save the "Virginia Creeper" in this country from the danger of becoming attacked by mildew, they should prohibit this importation or closely inspect the imported plants.

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BACTERIAL DISEASE OF *PISUM SATIVUM*.

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A SHORT preliminary note on this disease was published Nov. 1912(4), and since that date further investigations have been carried out into the life-history of the organism, and its effect on the host plant. As I there showed the disease is caused by a bacterium for which I propose the name *Pseudomonas seminum*.

As stated in the above mentioned preliminary note, the disease in this district, at all events, is a serious one, and now further details have come to hand showing that it occurs in other parts of the south of England. The disease causes considerable damage at this Institution where peas are grown fairly extensively for experimental purposes.

In the case of peas grown thickly in rows for culinary purposes, the diseased plants, unless they occur in large numbers, are either not noticed, or ignored, or their condition is attributed to one or more of the many diseases or insect pests to which eating peas are liable.

The most remarkable characteristic of this disease is that the organism is actually present in large numbers in the tissues of the cotyledon, and sometimes in the young embryo itself. Samples of diseased peas from Sleaford in Lincolnshire, sent to the Board of Agriculture in 1915 and forwarded to me for examination, proved to be infected with *Ps. seminum*, and showed the typical discoloration in the centre of the cotyledons. Other samples from various retail seedsmen have been examined from time to time, some of which have been absolutely healthy, others slightly diseased. The conclusion arrived at from the examination of these samples is that the disease is becoming more generally distributed. Whereas in 1913 and 1914 it was comparatively easy to find samples without disease, this year, 1916, considerable difficulty was experienced in procuring sterile seeds for experimental purposes, free from blemish inside or on the seed-coat.

Besides bacteria, *Sclerotinia* is very frequently present in the seed-coat, even after seed-sterilisation for 15 to 20 minutes in 1 per cent. corrosive sublimate. This treatment does not prevent germination in normal healthy seeds, but seeds attacked by *Sclerotinia* in many cases fail to germinate at all, or if they do germinate only make weak growth.

Such samples grown for culinary purposes under ordinary conditions in the open would give a good average crop, but so little is as yet known as to the external conditions controlling this disease, that a bad outbreak in any one year is not beyond the limits of possibility.

The disease occurs on both heavy and light soils. The earlier tall varieties of peas are much freer from disease than the later more succulent varieties. External temperature together with succulent growth greatly influences the development and spread of the organism in the plant.

The disease was especially bad during the first two or three summers after this Institution was opened, namely, 1910, 1911, and 1912. The land, which had previously been used as a market garden, is a heavy clay with an impervious subsoil of blue London clay. When the land was first taken over the drainage was quite inadequate and parts were liable to be under water in wet weather in the winter. Latterly, as the tilth has been improved by drainage, etc., the effects of the disease have not been so marked. In the case of a light soil, the previous history of which was known, the disease occurred on freshly broken poor grass-land, which had not carried a crop of peas for many years, if ever. This soil was somewhat deficient in lime.

In order to detect the presence of bacteria in the tissues, the young fresh succulent growth should be examined, where large numbers of the organism can be seen in the motile stage, in the cells of the cortical tissue near the phloem. As far as can be judged the organism penetrates and travels up through the plant in the motile stage, but it only remains motile if there is a considerable amount of moisture present.

Several instances of seeds with foreign organisms in their interior, both symbiotic and parasitic, have been recorded of recent years. The only strictly analogous case to this pea disease, in which however the parasite is a fungus instead of a bacterium, is that of Barley Smut (*Ustilago hordei nuda*), described by Broili and Schikorra in 1913[3]. These authors found the mycelium of the Smut both in the endosperm and the embryo of the resting seed. Another interesting point of similarity is that the fungus, during the earlier stages of the growth of the host plant, grows up with it in a state of semi-symbiosis without causing any very marked disturbance until the flower spike begins to develop.

On the other hand, in the case of the Rusts in the Gramineae, although Beauverie(2) observed sori of uredospores, teleutospores, and mycelium in the parenchymatous tissue of the caryopsis near the furrow, and in the pericarp beyond the region of the furrow, the mycelium could not penetrate the layer of strongly cuticularised tissue immediately outside the aleurone layer of the seed itself, unless this protective layer had been ruptured from some cause or other, in which case, bacteria were generally found to be present.

The most marked instance of a symbiotic bacterium inside the seed is that of *Ardisia crispa* and *A. crispa* var. *compacta* (Miehe(11)). The bacteria occur in the plant in nodules or pockets at regular intervals 30—50 mm. apart at a short distance from the edge of the leaf, in the buds and inside the seed between the embryo and the endosperm. Miehe considers this a case of strict symbiosis.

Instances of seed-coat parasitic infection and infection of the ovarial tissues are not uncommon. In the mycorhiza-bearing plants the mycelium of the symbiotic fungus in the Ericaceae was also found to be present in the seed-coat, but not in the internal tissues of the seed (Rayner(13)).

SYMPTOMS OF THE DISEASE.

The general symptoms are as follows: in mild cases, after germination, the shoot can develop normally, but in bad cases it is frequently abortive, brown, and dead at the tip, and laterals grow out prematurely to take the place of the main shoot (Plate V, fig. 1). Quite early in the development of the plant, when the plumule is from half an inch and upwards in length, light brown longitudinal streaks can sometimes be seen on the stem and root. In very bad cases little or no germination takes place. After this stage no further definite signs are noticeable till about the flowering period. Then the development of the disease depends a good deal on the external conditions. If the weather is warm and dry, and the plants are growing vigorously, the disease develops rapidly, and in a few days the plants may become unhealthy and change colour. The stem turns slightly brown and looks somewhat water-soaked. Brown longitudinal streaks appear at the base of the petioles on either side of the stem which is continuous with the midrib of the leaf. The streaks sometimes split open and dry out. The collar may be badly disorganised. The leaves become spotted and yellowish in colour, with darkened veins. Sometimes the surface of the pod is roughened or embossed. This is caused by the enlargement of cortical

cells, in proximity to other cortical cells filled with bacteria. If the disease is developing rapidly the younger portions of the plant show discoloration and fail to develop properly. Except in bad cases the plants can grow to full height, can flower and set a fair crop of seed, but on examination the cotyledons of the seed show the typical brown discoloured area in the centre of each cotyledon, which may be limited to a mere spot, or, on the other hand, nearly the whole cotyledon may be involved. The young embryo, especially the plumule, can also become infected. In bad cases there is frequently a cavity in the centre of each cotyledon. Sections of young green diseased cotyledons show large numbers of bacilli in various stages of development, both in the cells and in the intercellular spaces. If one pea is diseased all the other peas in the same pod are diseased to an equal extent. Healthy and diseased seeds have never been found in one and the same pod.

A large number of plants have been examined and the pods and seeds recorded in the order of development from below upwards, and it is a general rule that the first set pods show less discoloration of the internal tissues of the cotyledons than those developed later. Frequently the first set pod is free from infection. I venture to suggest in explanation of this, that the external temperature was too low for the rapid development of the organism, and the pods were able to mature sufficiently to resist infection before the organism could get the upper hand. The organism can only penetrate uninjured tissues when they are very young.

In some respects the symptoms resemble those of the formidable "Streak" disease of the Sweet Pea (*Lathyrus odoratus*) and in my preliminary note I suggested that Sweet Pea Streak was due to bacteria and not to *Thielavia basicola* as previously held (5,10). Early in the following year, 1913, a paper was read before the American Phytopathological Society, Cleveland, by J. J. Taubenhaus(9), and a short extract published in the *Gardeners' Chronicle*, showing that Streak in Sweet Peas was a bacterial disease due to *Bacillus lathyri*. This bacillus was found by the authors to occur on a wide range of other host plants, including Forage Lathyrus, Red Clover, Alsike Clover, Soy Bean, Wax Bean, Lima Bean, and Cowpea.

I have been unable to obtain further details than those published in this abstract, as to the biochemical characteristics of the organism, but a yellow organism isolated from a Sweet Pea suffering from Streak, which I took to be the same organism as that obtained by the above author, proved not to be *Ps. seminum*. Cross inoculations were

not tried, as Streak of Sweet Pea and bacterial disease of eating pea are both so prevalent at this Institution that the results would be wholly unreliable.

So far *Ps. seminum* has not been found on any other host plant; there is however no doubt that the organism occurs in the soil, as healthy peas sown on infected land can develop the disease. Judging from the fact that the organism can live a long time in artificial liquid media, it can probably live for years in the soil. To give one example of many records illustrating this capacity, a tube of sterile soil-extract was infected with a pure culture of *Ps. seminum*, and it grew well when plated after eight° months and two days. Old platings from other nutrient media gave similar results.

INFECTION OF THE HOST PLANT.

As mentioned above, the organism can only penetrate uninjured tissues when they are very young. Plate VI, fig. 1 shows the penetration of the epidermal cells of the young embryo of a pea germinated in sterile sand in a petri dish. It gains access into the tissues in the rod stage chiefly through the radial walls of the epidermal layer, but can also penetrate the young cuticle. The cell walls become swollen and disorganised (Plate VI, figs. 1, 2, S.W.) and the organism forces its way into the intercellular spaces and from there into the cells themselves. The nucleus is often attacked, the cytoplasm destroyed, and the cells dry out and rents occur in the tissues. These rents are particularly marked in dry weather and occur chiefly at the nodes, immediately below the base of the petiole of the leaf. Plate VI, fig. 2 shows the cells of the phloem parenchyma of a young stem, in which two cell walls have been broken down; and Plate VI, fig. 3 a cell of the cotyledon of a badly diseased pea, after germination. This last figure shows very clearly the penetration of the bacilli into the cells.

The organism occurs in the phloem, cambium, medullary rays, and occasionally in the pith, also in the parenchyma and vascular bundles of pods, in the tissues of the funicle, the cotyledons, and the leaves. It has no diastatic action on the pea starch. In Plate VI, fig. 3 a few starch grains can be seen intact, but most have been dissolved out by the chromacetic fixative. Unless some starch-dissolving fixative is used it is not possible to cut good microtome sections of the cotyledon. The bacilli have never been observed in the vessels. The occasional wilting of diseased plants is probably due to some other cause, such as

Fusarium or injury by soil insects. How the organism passes into the interior of the seed is not known. The bacilli have never actually been seen passing up the micropyle, but they occur in the thin-walled layer of cells lining the pod, immediately inside the sclerenchymatous layer of supportive tissue. The infection of the young seed probably occurs earlier, as all the seeds in a pod are diseased to an equal extent. It is more probable that infection takes place through the parenchyma and phloem of the vascular bundle which passes up the funicle, and which is connected with the vascular strands running along the midrib of the pod. Cultures of the organism, however, have been obtained from the cells of the thin-walled innermost·lining of the pod, which cells, under moist conditions, sometimes grow out into unicellular hairs.

The interior of a very young pea is filled with sap, and it was thought that the organism might be present in this sap, but smears made of this sap and stained with bacterial stains failed to reveal the organism in the rod stage.

MORPHOLOGY OF THE ORGANISM.

Ps. seminum is a large motile rod, with a single polar flagellum, attached rather to one side of the pole. The length of the rod varies considerably according to the age of the colony, and the medium used. On solid media, taken from a young colony the length is from 4μ to $5\mu \times 1\mu$, but in liquid media the rods can attain a length of from 9μ to 10μ before division. Chains of four to five rods reaching in length from 30μ to 40μ are not infrequently met with in young broth cultures. On solid media the organism soon passes into the beaded state. This consists of oval bodies, either separate or occurring in chains. They are formed inside the rods and are approximately as broad as the rods. They are highly refractive when alive, and might easily be mistaken for spores. When stained with ordinary bacterial stains such as Gram, Gentian Violet, Carbol Fuchsin, Methylene Blue, etc., these oval bodies take the stains very lightly. The margin is stained rather more than the interior, which remains almost colourless. As repeated efforts to stain them with spore stains failed, it was thought that the cultures were impure, and that there were two organisms present. In order to separate the two organisms numbers of sub-cultures were made in various liquid media including sterile water only, and then replated on to solid media, but without success. The oval and rod stages occurred persistently. Moreover, the colonies varied considerably in size and shape, all, however, giving the same characteristic reaction to litmus

milk. It was not until the development of the living oval bodies into the long rod stage was watched under the microscope that it could be definitely proved that both forms were stages of the same organism (see Plate VII, figs. 1—10).

A thin film of acid pea agar agar (1 per cent. normal HCl) was obtained by infecting the medium with a pure culture of *Ps. semmum*, and then before the medium had time to solidify, a sterile coverslip was dipped into it, so that only one side touched the medium. The cover-slip was then placed on a sterilised moist-chamber sunk slide, and fixed in position by two or three drops of paraffin wax. The slide was then placed upon a warm stage and kept under observation for twelve hours. Owing to the layer of air between the coverslip and the slide the dark ground illumination substage could not be used. However, by careful adjustment, it was possible to get a satisfactory lighting. Unfortunately, after twelve hours the coverslip was cracked while focusing, so that details of the beading stage, which, it will be noticed is just beginning in *f* group, Plate VII, fig. 10, could not be followed out. In a hanging drop the organism tends to become more and more difficult to see the older it grows. One rather interesting point to be noticed in these figures is that, when division is completed, the full-grown daughter rods come to lie parallel to one another instead of end to end, Plate VII, figs. 4—10.

This suggests that the daughter rods grow in length in the region somewhere near the point of division, especially as, in this case, the organism is growing on a solid agar medium, in which entire change of position would not be easy. The bending of the rod before complete division also rather supports this suggestion. In one case, *e*, it will be observed that complete separation has not occurred until the third division, and the rods lie end to end. It is only in fig. 8, that the marked bending appears in this group. Group *e* is not figured in figures 9 and 10 as it grew too large for the whole of it to appear within the field of the microscope, and also became overgrown by a neighbouring group outside the field. I venture to suggest that these observations, together with other factors, may account for the puzzling variability of the macroscopic appearance of the colonies. There were slight indications from time to time of the formation of thin capsules surrounding the rods, but with regard to this the lighting did not allow of any very accurate observations. These capsules, however, have been observed in stained sections. Smears of old cultures on solid media, stained for spores, do not give any very satisfactory results, as the organism gets massed together

without any very clear definition. The best spore-staining results were obtained from pellicles in liquid media and from growths on potato. The spores can be seen, Plate VII, fig. 14, in the middle or to one side of the oval bodies, so that they appear to protrude. The surrounding substance of the oval bodies seems to swell gradually and become disintegrated so that the spores eventually lie embedded in a pseudo-zooglaeal mass.

I consider the oval bodies to be a kind of involution form as they sometimes vary in size and shape. The germination of spores has not been observed. It is difficult to identify the organism in microtome sections of the older parts of the plants, or in the dry cotyledons, owing to the oval bodies and the pseudo-zooglaeal masses. They can hardly be distinguished from the surrounding heavily stained disorganised tissues of the host plant. In the case of dried peas it is only after they have been kept under moist sterile conditions conducive to germination from 10—14 days, or even longer, that the rod stage can be definitely seen. In hand sections of the young succulent parts of the plants, the rods can be readily seen moving about in the cortical cells, although the tissues appear healthy and turgid. The organism can be isolated with ease from the centre of the cotyledons and from the diseased stems. If sufficient care is taken, almost pure cultures can be obtained by infecting tubes of nutrient broth with a small portion of the tissues of the stem or cotyledons, although in the case of cultures from the dry cotyledons the culture tubes have to be kept in the incubator at least 10 days before a satisfactory plating can be obtained. The organism grows well at a temperature of 25° C.

VARIABILITY IN GENERAL CHARACTERISTICS OF COLONY.

Differences in macroscopic appearance in cultures on artificial media do not necessarily mean impurities. Capsular organisms, or those which produce slime, may develop, and mostly do develop, giant colonies(1). Individuals get stuck together, but on the other hand there is always the danger of foreign organisms being caught up in the slime which may prove to be very difficult to separate out. Marshall Ward(14) in 1895 maintained that variation in form, rate of growth, and other characteristics of plate colonies result from much slighter variations in the medium and other environmental conditions than is generally recognised: also, that organisms are affected by the vicissitudes to which they have been subjected previous to culture. First platings of the wine bacterium as observed by A. J. Perold(12)

gave colonies of different appearance, but later platings showed uniformly pure cultures. Among others *Bacillus mycoides*(6), *Bacillus megatherium*(7), are instances of organisms non-pathogenic to man, which show considerable colony variability.

Ps. seminum also shows considerable variability in size and general characteristics of its colonies on artificial media. The fact that the organism produces a thin capsule round the rods when they come to rest after the motile stage, and has its spores embedded in a kind of matrix, may partly account for the variability shown. In liquid media it hangs together in long chains, and as I have already pointed out, this phenomenon of incomplete division can also occur on solid media, and may be a possible explanation of the occurrence of straggling outgrowths in the submerged, and lobing or radiations in the surface colonies.

The two forms of colonies most frequently met with in platings of *Ps. seminum* are (1) surface opaque white or whitey-buff, more or less circular, and (2) surface less opaque, more or less widely radiating colonies, with their corresponding lens-shaped and straggling submerged colonies (Plate VII, fig. 13). Repeated tests as to their reaction to litmus milk showed identical results with both colonies. They also give the same reaction with sugars. There is no doubt that the amount of water in the medium greatly influences the size and length of the lobes or radiations in the spreading colonies. Treatment of the organism previous to plating may also have some effect. If the bacillus is isolated from the young green cotyledon or the growing stem, it tends to throw spreading colonies; whereas more or less circular surface colonies result from young cultures from dry cotyledons incubated in peptone beef broths. If pure cultures of first platings are kept for some time in liquid media, so that a pellicle or flocculent sediment is formed, the resulting colonies then vary considerably from the original colony.

To give illustrations of this, a tube of alkaline (1 per cent. normal NaOH) peptone beef broth was infected with a surface opaque whitish circular colony from a dry cotyledon, and, replated after five months and twelve days on acid (1 per cent. normal HCl) pea agar agar, gave a straggling surface colony. On the other hand a tube of sterile soil extract was infected with a straggling colony and replated on neutral pea agar agar, and gave the following:

(1) More or less circular opaque colonies, inclined to be lobed.

(2) One large semi-transparent spreading colony which almost completely covered the whole surface of the petri dish.

(3) Radiating semi-opaque colonies.

(4) Submerged colonies, flat disks with roundish lenses in the periphery (Plate VII, fig. 12).

This plate to all external appearances looked impure, but careful tests of all these four types of colonies gave identical and typical reactions with litmus milk. A good deal also depends upon the morphological stage of the organism at the time of plating. Replatings from one solid medium to another tend to retain their original colony characteristics, but ringing the changes between solid and liquid media only led to further puzzling results and occasional impurities.

The inference to be drawn from these facts is that there are possibly two strains of *Ps. seminum* which differ only in their shape of colony. One strain may be more capable of complete division than the other. The proportion of definite rods to oval bodies is greater in the radiating colonies than in the circular surface colonies.

Platings from young cultures and from very old cultures in liquid nutrient media, give much more uniform results than platings from the intermediate stages. Also platings of the pellicle are naturally much more varied than when the pellicle is avoided and platings made from the broth itself. The colonies also vary in general characteristics according whether the medium is acid, alkaline, or neutral (Plate V, figs. 3, 4, 5) although the organism grows well on all three. Plate V, figs. 3 and 4 show magnifications of giant colonies of the same culture on neutral and alkaline media, and it will be seen that on neutral pea agar agar the individual submerged colonies are much more definitely lens-shaped than on the alkaline medium.

Plate V, fig. 5 also shows the different growths although the platings are rather thick, and Plate VII, fig. 12, drawings of varying shapes in submerged colonies, all of which give the typical litmus milk reactions.

BIOCHEMICAL REACTIONS.

Ps. seminum is a facultative anaerobe and will grow to the bottom of a closed agar stab.

Beef broth. The organism grows well on acid (1 per cent. normal HCl), neutral and alkaline (1 per cent. normal NaOH) broth. A pellicle is formed, which, on shaking, sinks to the bottom of the liquid and forms a stringy precipitate. This precipitate gradually disappears and the liquid becomes turbid. Both oval bodies and rods strung together in long chains occur in the pellicle, but if the latter is avoided, rods and

no oval bodies show in smears made of the liquid itself. Most pellicle occurs on alkaline beef broth.

Potato. The organism grows well on sterile potato chunks. The growth forms a layer of creamy consistency over the surface, and becomes contoured or creased when the potato begins to dry out. There is no discoloration, but occasionally a pinkish tinge appears in the bacterial growth both on potato and on the testa of the pea seed. Plate V, fig. 2 shows a two-year old pea enlarged, which was sterilised in 1 per cent. corrosive sublimate for 15 minutes, having been previously moistened in sterile water under reduced pressure. It was then placed in sterile sand to germinate. There was, however, no germination. The seed was dead, but a prolific contoured bacterial growth developed on the testa, slightly pinkish in tinge, which proved to be a pure growth of *Ps. seminum*. Microtome sections of the testa covered with bacterial growth showed masses of long rods and oval bodies embedded in a matrix, composed partly of disorganising rods.

Gelatine. Gelatine is rapidly liquefied. The liquefaction in a stab is elongated napiform at first, but finally the whole of the gelatine in the tube is liquefied. A pellicle and precipitate are formed as in the peptone beef broths. There is no odour with any of the artificial media.

Sugar reactions. Stabs made in litmus gelatine with 1 per cent. dextrose, saccharose, and lactose respectively show liquefaction and acid formation with dextrose and saccharose, but only liquefaction and no acid with lactose. The development with lactose is slower than with the other two above mentioned sugars. No gas is formed with any of the sugars.

Litmus milk. The reaction with litmus milk is very characteristic. The litmus is slowly reduced without the formation of acid, and passes gradually through a greyish tinge to a clear apricot colour. The shade of apricot varies slightly according to whether the original litmus is pale and alkaline, or darker and rather on the acid side. In the latter case the apricot colour is rather darker. There is no coagulation, and the milk gradually becomes quite clear, with the usual bacterial sediment. This apricot reaction is very typical of *Ps. seminum*. Both spreading and circular colonies give exactly the same reaction. If the tube is shaken when the clear apricot colour has formed, the liquid turns pinkish in colour in a few minutes, but the clear apricot colour returns after a few hours. This pinkish colour may also appear if the tubes are taken out of the incubator and cooled down to about 14° C., but the apricot coloration returns when the cultures are replaced in

the incubator. This pinkish colour cannot be due to acid, as all the blue coloration of the reduced litmus has disappeared. The colour then changes from apricot to deep sherry, and finally the liquid is a clear, deep wine shade with a heavy bacterial sediment. The action on litmus milk is slow.

Glycerine. There is good growth on glycerine without the formation of gas or acid.

Nitrate reduction. Nitrate is reduced to nitrite without the formation of gas. The organism soon loses its power of reducing nitrates when grown on artificial media. Only young cultures from the growing stem and freshly incubated cultures from the dry cotyledon should be used for this test. The organism however retains its power of reducing litmus a very much longer time. The apricot reaction occurs with cultures some months old. The nitrite test used was that recommended by Jordan(8), namely:

After four days incubation in nitrate broth (0·1 per cent. peptone, 0·02 per cent. nitrite-free potassium nitrate) add to 3 c.c. of the culture 2 c.c. of each of the following:

(1) Sulphanilic acid solution made by dissolving 8 grammes of the purest sulphanilic acid to 1000 c.c. of 5N acetic acid (sp. gr. 1·041).

(2) α-Amidonaphthalene acetate solution prepared by dissolving 5 grammes solid α-Naphthylamine in 1000 c.c. of 5N acetic acid and filtering through absorbent cotton wool.

The development of a rose colour indicates the presence of nitrites.

Diastatic action. The organism has no diastatic action on pea starch.

Stab Cultures. The line of puncture is indefinitely beaded and growth occurs down to the bottom of a closed agar stab.

Reactions to stains. In the rod stage *Ps. seminum* takes the usual bacterial stains very readily. It is gram-positive and non-acid fast. But as already stated above the oval bodies only stain lightly. The flagellae are also very difficult to stain; the best results were obtained with van Ermengen's flagella stain.

Isolation of the organism. The organism has been isolated from the centre of both green and dry cotyledons, from the stem, from the pod, and from the hair-like outgrowths sometimes found lining the pod.

Motility. When the parent rod is about to divide, it has a slow sinuous motion, but when division is completed, the daughter rods have been seen at first to revolve very rapidly on their own axes, as indicated by the arrows in Plate VII, fig. 14 a, and then to dart about the field of the microscope. When observed alive in the cells of the

host plant the general movement is slow, with an occasional swiftly moving rod.

Group number. The group number of this organism, according to the Description Chart of the Society of American Bacteriologists is:

<center>*Ps.* 121·2323033.</center>

So far as is known to the author, this organism has not been previously described, and has therefore been named *Pseudomonas seminum.*

<center>INOCULATION EXPERIMENTS.</center>

Inoculation experiments were carried out in the open, but little stress can be laid upon the results, as the disease was so prevalent throughout the experimental plots. Pea plants grown in boxes in soil partially sterilized by heat and inoculated just above the ground when the plants were one foot high, showed no disease, whereas in the open seven out of ten inoculations in the stem below the youngest leaf were successful. These contradictory results were probably due to the part of the plant inoculated. The stems of the plants in the boxes had probably matured sufficiently to enable them to withstand the spread of the organism.

Other outdoor experiments were tried in which the young unfolded leaves were pressed apart near the growing point, the young stem pricked with a sterile needle, a drop of sterile water containing a pure culture of the organism placed on the wounded stem, and the young leaves replaced over the drop. These inoculations also gave definite results in so far as the growth was partially arrested, the leaves turned yellowish, and the upper part of the plants showed the general symptoms of the disease. However, in this case also, some of the control plants treated in the same way with sterile water only, developed the disease. Further attempts were made to grow pea plants under sterile conditions in sand in carboys and watered with nutrient salt solutions, but unfortunately the cultures did not remain sterile owing to the necessity of opening the carboys for manipulation. The seeds for these experiments were sterilized with 1 per cent. corrosive sublimate for twenty minutes, washed twice in sterile water and then placed singly in sterile petri dishes to germinate. After germination the testa was removed, the cotyledons of the seeds were forced apart with sterile instruments to see if the internal tissues were healthy, and those showing any blemishes on any part of the seed were rejected. When the first leaves had unfolded, the peas were planted in the

carboys, which were kept lying on one side throughout the experiment, great care being taken to keep them as sterile as possible during manipulation. Five days after the plants had been placed in the carboys they were inoculated with a pure culture, in sterilized water, of a surface more or less circular opaque whitish colony isolated from the centre of a diseased cotyledon. A drop of the culture was placed on a young leaf. The leaf turned browny yellow in three days, at the end of ten days the growing point of the stem was practically dead, and laterals had begun to develop. Identical results were obtained from a radiating colony, see Plate VII, fig. 13, from the same original culture, whereas the controls, treated with sterile water only, remained healthy throughout this period. Another carboy was inoculated with a mixture of the two above-mentioned colonies with exactly the same results. The main shoot died back in 10—11 days. These results are only conclusive in so far as they show that young tissues can be infected and die back when inoculated on the surface with a pure culture of *Ps. seminum*.

Pea plants grown under sterile conditions make poor growth, even when supplied with a complete nutritive solution and, as stated above, succulent growth is necessary for the full development and rapid spread of the organism in the plant. It is also difficult to obtain seed from pea plants grown under moist sterile conditions. Nearly all the flowers in the above experiments shrivelled and dropped off.

<center>SUSCEPTIBILITY.</center>

All attempts to find an immune variety of pea have been without success. A number of different varieties of mid-season and late culinary peas were grown to test their resistance to the disease, but all varieties, so far, have been found to be more or less susceptible. The variety which proved to be most resistant was Sutton's Improved Petit Pois, which, sown on infected land, gave from 49 to 50 per cent. healthy plants. Seeds saved from these selected healthy plants and sown again on the same plot the following year also gave approximately 50 per cent. healthy plants.

Duke of Albany and Ne Plus Ultra are very susceptible, the average number of healthy plants of Duke of Albany being 14 to 15 per cent. The growing of the Ne Plus Ultra for Mendelian experiments at this Institution had to be discontinued owing to its extreme susceptibility.

As mentioned above, the taller varieties of early peas show considerably less disease than the later varieties, partly, it is believed, because of their less succulent growth and earlier development, which

latter enables the plant to attain sufficient maturity before external conditions are conducive to the rapid development of the organism.

Thanks to the courtesy of Mr F. J. Chittenden, I was able to examine a large number of different varieties of early peas grown at the Wisley Early Pea trials in 1915. The plants had all but ripened off, and only one variety was found which showed the typical discoloration of the cotyledons, although, judging from external appearances there were many doubtful cases. A row of Duke of Albany, growing in an adjacent plot but in a much earlier stage of development, showed the typical discoloured patch in the centre of the cotyledons.

It must, however, be pointed out that the disease is not so prevalent at Wisley as it was at this Institution, and that the trials, so far as the disease is concerned, cannot be taken as giving conclusive results. But general observations on pea plants grown on soil known to be infected illustrate this point much more conclusively. There is also the possibility that, in the two cases in point, of diseased varieties occurring at Wisley the disease may have been introduced with the seed, as it is impossible to tell from the external appearance whether a seed is healthy or diseased. It can thus be seen that it is very difficult to get results which are really reliable.

Of the dwarf succulent early varieties Chelsea Gem and Little Marvel, and of the later varieties Ne Plus Ultra and Duke of Albany, are particularly susceptible. Sugar peas and rogue peas can also become badly diseased when grown on infected land.

It is hoped, after further trials, that it may yet be possible to find an immune variety, so that by breeding this immunity may be transferred to some of the excellent varieties of culinary peas already on the market.

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REMEDIES.

So far no cure is known: in fact, a cure is hardly possible in this case of a succulent annual, especially when the seed itself may be infected with the parasitic organism. The precautions to be recommended are the rotation of crops, early sowing, and general healthy cultural conditions as to drainage, sufficiency of lime, etc., and the rejection, where possible, of seeds known to have come from diseased plants. The haulms from an infected crop, together with the sticks, should be burnt as soon as possible after the crop has been gathered. Partial sterilisation of the soil is useless, as *Ps. seminum* is a sporing organism; it is also impracticable owing to the amount of labour required

and the expense. Care should be taken to thoroughly clean all implements used on infected land so as not to spread infection.

SUMMARY.

Ps. seminum is a motile sporing facultatively anaerobic bacillus capable of growth on acid, neutral and alkaline media.

The chief characteristics of the disease caused by this organism is that it is present in the interior of the seed, forming a discoloured area in the centre of each cotyledon. It is not possible to tell from the exterior of the seed whether it is, or is not, infected.

External infection takes place through the young tissues, and, as far as is known, the organism is unable to spread through matured tissues.

Infection occurs considerably in advance of discoloration in the tissues.

The organism occurs throughout the plant, but has never been observed in the vessels.

It has been isolated from the interior of the seed, both dry and green, the stem, the embossed pod and the layer of thin-celled tissue which forms the innermost lining of the pod.

The organism has never been seen passing through the micropyle of the seed, although on one occasion rods were observed lying in the spaces between the cotyledons and the young embryo.

The organism passes up the plant in the motile rod stage.

The macroscopic appearance of the colonies on artificial media is very variable.

The organism develops rapidly at a temperature of 25° C. but development is slow below 20° C.

The motile stage only lasts a short time unless there is a considerable amount of moisture present. This is followed by the beaded stage, and finally spores are developed inside the beaded stage, and the spores eventually lie embedded in a pseudo-zooglaeal matrix.

All varieties of culinary peas so far examined are more or less susceptible.

Germination is not arrested, but in bad cases the development of the plant is retarded owing to the dying back of the main shoot and the development of laterals to carry on the growth of the plant.

DESCRIPTION OF PLATES.

PLATE IV.

Fig. 1. Peas germinated in a cool greenhouse in soil partially sterilized by heat.
1, 1, 2, 2. Diseased seed.
5. Control, healthy seed.

Fig. 2. A. Healthy plant, just before flowering period.
B. Four diseased plants of the same age.

PLATE V.

Fig. 1. A. Seven diseased seeds germinated in sterile sand in a petri dish.
B. Typical healthy seedling grown in a pot.

Fig. 2. Pea seed enlarged, two years old, kept in sterile sand to germinate. No germination. The contoured growth (a) on the testa is a pure growth of *Ps. seminum.*
(b) Grains of sand still adhering.

Fig. 3. Giant colonies of *Ps. seminum* enlarged, growing on neutral pea agar agar.
(a) Submerged colony.
(b) Surface colony.

Fig. 4. Same culture as in Fig. 3, but growing on alkaline pea agar agar.
(a) Submerged colonies.
(b) Surface colony.

Fig. 5. Platings of *Ps. seminum.*
(a) Acid (1 per cent. normal HCl) pea agar agar.
(b) Alkaline (1 per cent. normal NaOH) pea agar agar.
(c) Neutral pea agar agar.

PLATE VI.

Fig. 1. Microtome section of epidermal cells of young embryo of pea germinated in sterile sand, showing penetration of bacteria into uninjured cells and into the intercellular spaces.
S.W. Swollen cell wall.
I. Intercellular space, filled with bacteria, stained Carbol Fuchsin and Licht Grün.

Fig. 2. Microtome section of phloem parenchyma of young diseased stem infected with *Ps. seminum.*
S.W. Swollen disorganised cell wall.
B. Bacteria.
H Healthy cell wall

Fig. 3. Microtome section of cotyledon of pea germinated in sterile sand.
B. Bacteria.
D. Disorganised cell walls.
H. Healthy cell wall.
I. Intercellular spaces filled with bacteria.
S. Starch grains.

PLATE VII.

Growth of *Ps. seminum* during a period of twelve hours, on a film of acid (1 per cent. normal HCl) pea agar agar.

Fig. 1.   Oval bodies, 12.20 p.m.

Fig. 2.   3.10 p.m.   Rods developed from the oval bodies.

Fig. 3.   3.40 p.m.

Fig. 4.   3.55 p.m.   Complete division occurred in groups *a* and *f*.

Fig. 5.   4.25 p.m.   Second division beginning in *a* group, parallel position in *f* group. Complete division in *c* group and beginning of parallel position.

Fig. 6.   5.25 p.m.   Third division in *a* group.   Parallel positions in *c* group after second division.   Incomplete division in *e* group.   Growth beginning in *g*.

Fig. 7.   6.30 p.m.   Still incomplete division in *e* group.   Development in *g*.   Parallel growth in *d* and *f* groups.

Fig. 8.   7.30 p.m.   Complete division in *e* group for the first time, after four subdivisions.   Parallel growth in *b* group.

Fig. 9.   9.5 p.m.   Division occurring in *g* group.   Parallel growths in *a, b, f, d* groups. One very long rod in *c* group.

Fig. 10.   11.50 p.m.   Complete division and beginning of parallel growth in *g* group. Beading showing in *f* group.

Fig. 11.   Flagellae of *Ps. seminum*.

Fig. 12.   Different types of submerged colonies of *Ps. seminum* grown on different media.
    (a)   Submerged colony on acid pea agar.
    (b), (c), (d).   Submerged colonies on alkaline pea agar.
    (e), (f).   Submerged colonies from green peas on acid pea agar.
    (g)   Submerged colony on neutral pea agar.

Fig. 13.   (h) (h) Spreading surface colonies.
    (i) (i) Round surface colonies, on acid pea agar agar, plated after three months' incubation of a piece of the centre of the cotyledon of a dry pea.

Fig. 14.   Showing different stages of *Ps. seminum*.   Note capsules, oval bodies of various shapes, and spores.

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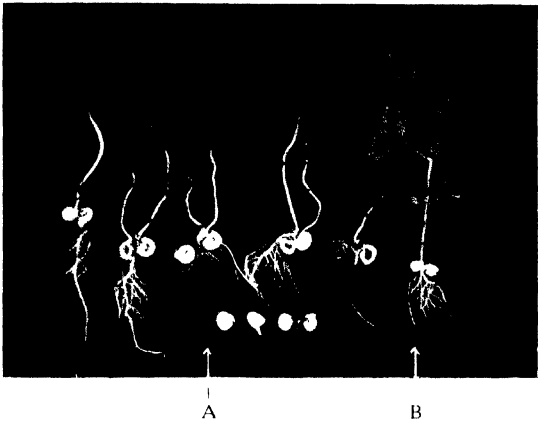
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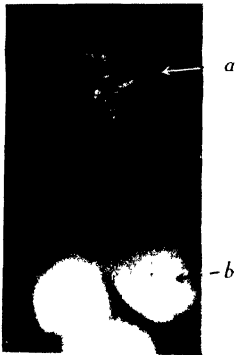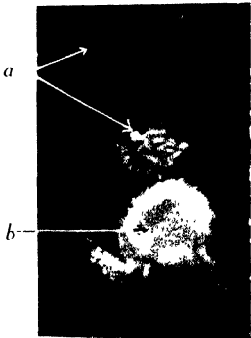

Fig 3.

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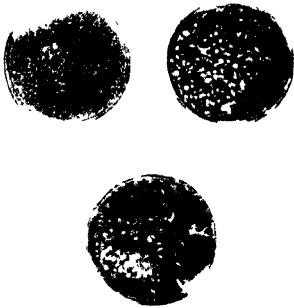
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Fig 2

Fig 1
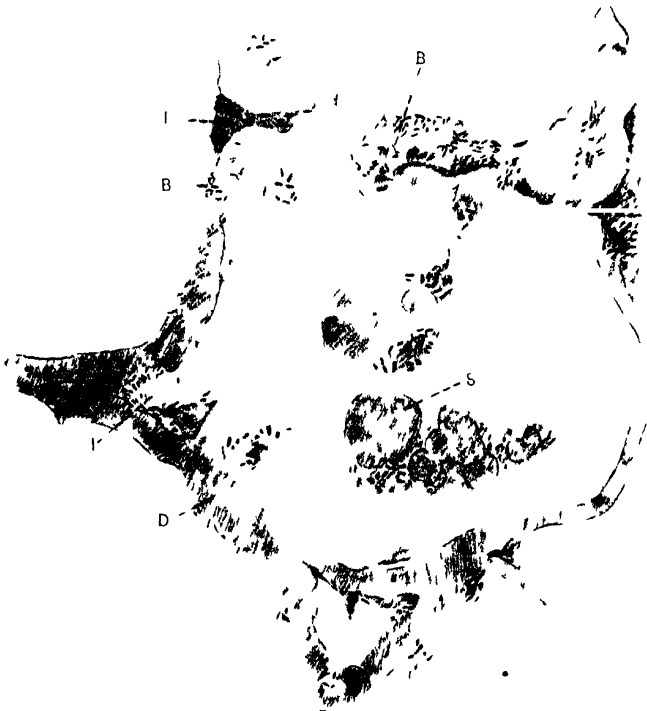
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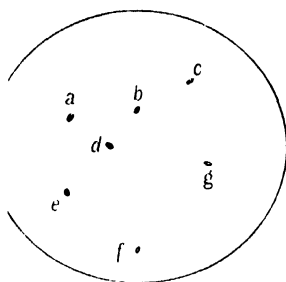
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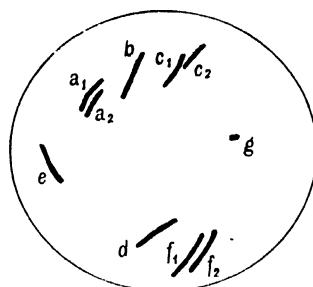
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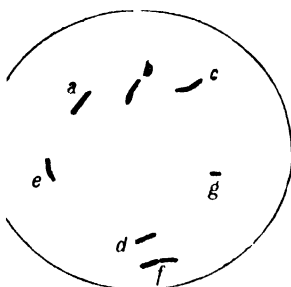
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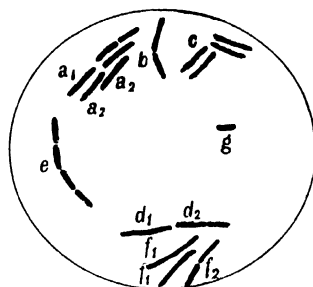
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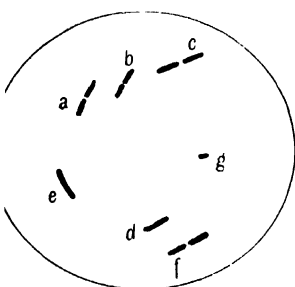
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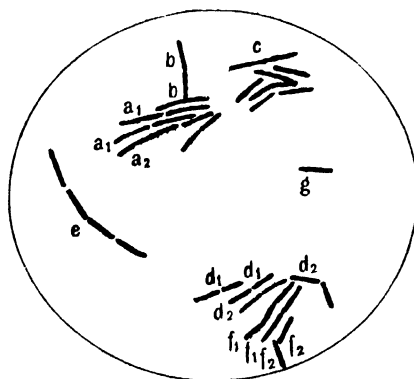
Fig. 7.   6.30 p.m.

Fig. 4.   3.55 p.m.

PLATE VII
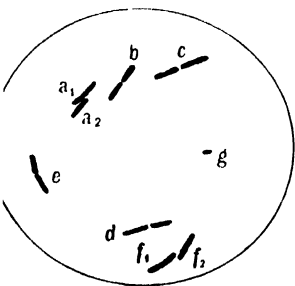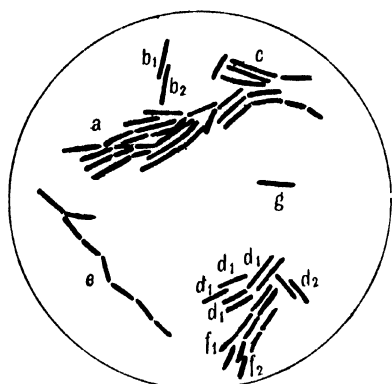
Fig 8.   7.30 p.m.
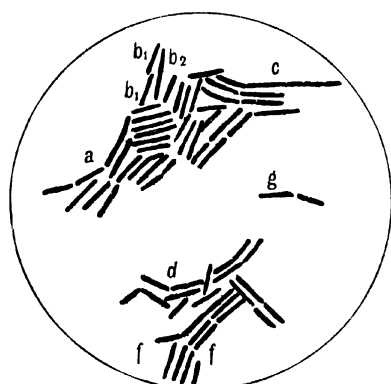
Fig. 9.   9 5 p.m.
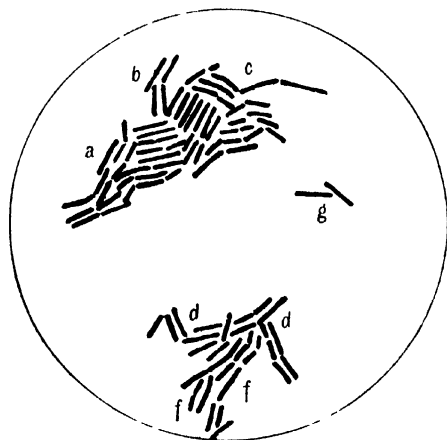
Fig 10.   11.50 p.m.
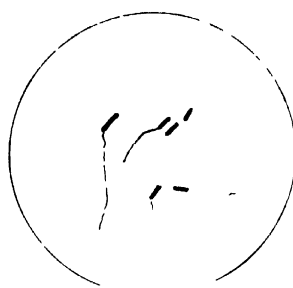
Fig. 11.
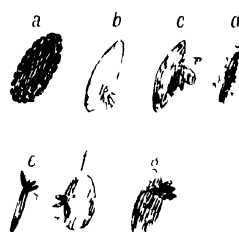
Fig 12.

Fig. 13.
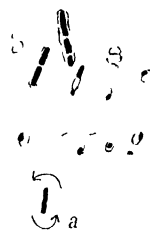
Fig. 14.

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STUDIES IN BACTERIOSIS.

1. "BLACKLEG" OF THE POTATO. ·

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INTRODUCTION.

THE losses caused by bacteriosis of the potato in this country are
fortunately not so great as they appear to be in other parts of Europe,
the United States, and in Canada. Compared with fungal diseases
those of bacterial origin are quite of secondary importance and do not
usually cause any serious trouble. In most districts a certain number of
plants succumb each year to attacks of bacteria but they are so few that
very little notice is taken of them, they are usually pulled up and burnt,
but sometimes are left standing in the field without detriment to the
neighbouring plants. The chief losses are incurred during storage in
the *clamp* or *pie*, and unless special precautions to insure a good
system of ventilation are taken in building the clamp the conditions
of warmth and moisture may become so favourable to the growth
of bacteria that a few diseased tubers included in the crop act as foci
from which disease spreads. In this way the whole, or a large part,
of the store occasionally becomes involved. If on any field a more
than usual number of plants showing signs of bacterial disease are
noticed during the summer the wise man takes the precaution not to
store the crop from this field but to send it at once to market.

Epidemics are of such rare occurrence in this country that the
subject has received but little attention by English pathologists. In
Canada however the losses from this cause have assumed much more
serious proportions, Harrison[6] found the average percentage of rotted
tubers during three years as high as 22 per cent., and the report of the
crop correspondent of the Canadian Bureau of Industries for 1905

shows losses from rot ranging from 10 per cent. to 75 per cent. In Galicia also the percentage of diseased plants was very high during 1907—1910; von Hegyi[8] reported that 40 per cent. to 60 per cent. of the crop was attacked in some experiments in which "seed" from a badly infected area was used. Although the extent of loss of potatoes by bacterial disease is at present low, probably not more than 5 per cent. of the entire crop of Great Britain, it may at any time become much more serious, and in fact there are indications that it is steadily on the increase. The Board of Agriculture[21] recognises two bacterial diseases of the potato namely *Blackleg* and *Brown Rot*; these have been identified by the outward symptoms and the presence of bacteria in the tissues, but the actual isolation and identification of the causal organisms has not been attempted. It seems therefore desirable that a thorough investigation of these diseases should be carried out and the present paper embodies the results of a study of the former disease as it occurs in Lancashire.

SYMPTOMS OF THE DISEASE.

The symptoms of the disease known as "Blackleg" have been described very fully by Appel[1] and more recently by Pethybridge and Murphy[15] so that it is unnecessary to give more than a brief description of the general characters of the disease. It usually makes its appearance early in the summer during June or July, and especially when a spell of hot weather follows a rainy period. On looking across an infected patch of potatoes one sees here and there a plant distinguished from the rest by the wilted and yellow appearance of its leaves; these later become dark brown or almost black and very much shrivelled. On close inspection the stem shows at the ground level a blackened area which gradually spreads upwards as the disease progresses. Such stems when pulled gently leave the soil with scarcely any resistance, there being as a rule an entire absence of development of tubers. The pith at the base of such stems is completely rotted away leaving a hollow space surrounded by a more or less healthy cortex in which the vascular bundles stand out very prominently in virtue of a strong brown pigment in the walls of the vessels. If the attack has occurred late in the summer the disease will have spread from the main stem through the underground stems to the developing young tubers, and if these are cut longitudinally through the "heel" they show the vascular ring marked out by the same brown pigment which stains the vascular bundles of the stem. In many cases this

browning is limited to the bundles at the "heel" end only; this is usually the only sign of disease exhibited in the young tuber, the storage tissue having quite a normal appearance. Microscopic examination of a section across one of these bundles shows the vessels filled with a mass of bacteria : from the vascular bundles the organism under suitable conditions rapidly invades the starchy tissue' reducing it to a soft pulpy mass with very offensive smell. Potatoes in such a condition are frequently found in the field, especially if rain be followed by a hot baking sun. In the important potato-growing districts of Lancashire these tubers, thus rotted in the soil, are locally termed "*par-boiled.*" Microscopic examination of the pulpy mass reveals the cells separated from one another, the substance of the middle lamella being apparently dissolved by an enzyme; the starch grains appear to be quite unaltered.

The propagation of the disease is usually assumed to be due to the planting of diseased "sets"; von Hegyi[8] however who investigated "Blackleg" in Galicia and Prussian Silesia has stated that in every case the "blacklegged" shoots examined by him bore evidence of the attack of *wire-worms*, and that biting insects are a necessary factor for the entry of the parasite into the host. The fact that the parent "set" from which the diseased shoots have arisen will almost invariably be found to have decayed points to the planting of diseased "sets" as the source of the disease in most cases. This is further emphasised by the fact that the disease makes its appearance in isolated individuals and is only rarely to be found affecting even small patches. The general lack of anything in the nature of an epidemic of "Blackleg" spreading from a certain focus of infection would seem to indicate that propagation by wire-worms is not usual, though undoubtedly biting insects may be instrumental under certain conditions in introducing the parasite from the soil. Morse[14] gives one instance of such an epidemic spreading over a patch of potatoes where the soil was exceptionally moist, and it is probable that insects were the responsible agents in this case.

ETIOLOGY.

The disease known as "Blackleg" appears to be caused by a number of different parasites. *B. phytophthorus* was isolated and named in 1903 by O. Appel[1] in Germany and the same organism has been found by E. F. Smith[18] and L. R. Jones[10] as causing the disease in America. In 1911 Pethybridge and Murphy[15] described the disease under the name of "Black Stalk Rot." The organism which they isolated from diseased plants in Ireland differs only slightly from that

described by Appel, and they suggest that it is probably only a variety of the same organism, but since it differs constantly in certain particulars they rather reluctantly formed a new species *B. melanogenes*. The disease investigated by F. C. Harrison[6] in Ontario resembles in outward characteristics that of "Blackleg," as described by Appel, except that while in the latter the woody bundles remain hard and strong enough to support the diseased shoot so that these are conspicuous objects standing erect in the field, in the former the vascular bundles are so softened that the wilted and blackened shoots fall to the ground and lie hidden so that on casual inspection the crop has the appearance of being perfectly healthy.

Mention must be made here also of a rotting disease of the potato ascribed by E. F. Smith[19] to *B. solanacearum* since the final wilted appearance of the shoots might be mistaken for the symptom of "Blackleg." In this disease however the progression of the wilt is from above downwards, the infection occurring in the leaves through the agency of leaf-biting insects. while in "Blackleg" the lower leaves are the first to show signs of wilting since the point of infection is subterranean. Besides these a number of other organisms have from time to time been described as producing rot in the potato accompanied by the appearance of "blacklegged" shoots. Thus we find *B. atrosepticus* described by van Hall[5], *Micrococcus phytophthorus* by Frank[3], *B. caulivorus* by Prillieux and Delacroix[16] and *B. solanincola* by Delacroix[2].

Of these *Micrococcus phytophthorus* was possibly not the cause of the disease which Frank had before him. Various workers have stated that Micrococci appear abundantly on plate cultures from diseased stems but that these prove to be saprophytic organisms. Frank describes a special resistance to rotting exhibited by the potato in winter. He states that his cultures during September and October produced rapid rotting while those of December and January. had no pathogenic properties. While it is quite conceivable that physiological changes in the tuber might result in such a resistance it seems more probable, as Appel suggests[1], in view of the frequent occurrence of saprophytic Micrococci, that Frank was dealing with mixed cultures probably containing *B. phytophthorus* in which a saprophytic Micrococcus predominated, and that by December when the "special resistance" made its appearance the real parasite had been lost. *B. caulivorus* was considered by Laurent[12] and Griffon[4] to be identical with *B. fluorescens liquefaciens*. Both hold that under certain conditions of the soil common

saprophytes may take on the characteristics of parasites and produce disease in plants. Laurent holds an alkaline condition of the soil to be the essential factor, while Griffon believes that atmospheric humidity, moist soil and a variety of potato of low resistance are the necessary factors which induce rotting by *B. fluorescens.* Neither Laurent nor Griffon appears to have made convincing infection experiments with *B. fluorescens*, the latter in fact states that his attempts in this direction were not encouraging, and as Riehm points out in an abstract of Griffon's paper(17) when the author shows the presence of this organism in plants which are decomposed he has no proof of the pathogenicity of the bacillus. The question whether bacilli of the fluorescent type are capable of producing disease in the potato is thus in some considerable doubt. Jensen(9) brought evidence to show that many ammonia-producing organisms may enter living plants through wounds in virtue of the lethal effect of ammonia upon the cells, so that dead material continually presents itself to the invading organism.

Doubt has also been raised by E. F. Smith(20) as to the pathogenicity of the organism described by Delacroix under the name *B. solanincola.* A culture of this organism obtained by Smith was not virulent, and an examination by him of the material left by Delacroix as typical of the disease revealed no bacteria in the vessels; on the other hand there was considerable evidence that fungal parasites had been the cause of the disease. Delacroix's description of the disease seems to show that it had a bacterial origin and it would be unsafe to place too much weight upon the evidence to the contrary which Smith's research has produced, since the material to which he had access might not have been quite typical of that examined by Delacroix and the culture might have lost the virulence which it had once possessed.

We have then four or five different organisms which have been described in other countries as giving rise to the symptoms of "Blackleg" but up to the present no one has fully identified the cause of the disease in Great Britain, hence earlier statements by Massee(13) and others to the effect that it is due to *B. phytophthorus* are not supported by the evidence at present available. This work was undertaken with the purpose of determining which of the several organisms are present in this country. Since this paper was written a publication by Morse(22) has come to hand in which it is shown that three of these organisms, namely *B. atrosepticus, B. solanisaprus* and *B. melanogenes*, when cultivated under the same conditions give identical physiological reactions and possess morphological characters which differ so slightly

that, on these alone, a separation into distinct species is not warranted. These must therefore be considered as strains of one species to which Morse applies, on grounds of priority, the name *B. atrosepticus*.

COLLECTION OF THE MATERIAL.

During the months of summer when "Blackleg" makes its appearance the weather in 1916 was particularly dry over most parts of England so there was perhaps less of this disease than is normally the case. However when this investigation was begun in the second week in August it was reported that there had been rather a considerable amount of it in certain parts of Wiltshire and Devonshire and also in parts of Surrey and Kent. The material investigated was collected during a survey of Wart Disease in the Ormskirk District of Lancashire. Stalks showing the symptoms of "Blackleg" were .not numerous, in fact on many fields they were difficult to find. This was mainly due to the fact that the weather had been particularly dry for some weeks. Several diseased stalks were pulled up here and there and cultures were made the same night by inoculating from the most recently diseased portions of the pith upon slopes of potato-mush-agar. On the day of leaving the district a plot some twelve drills wide and of about two acres area was found in which the percentage of "blacklegged" stalks was very high, about one plant in six showing the disease. This plot had no pegs or other marks to distinguish it from the neighbouring plots but the appearance of "Blackleg" upon it was so marked that its limits could be most distinctly seen on looking down the drills. Search was made on a neighbouring plot but not a single case of a "blacklegged" shoot could be found. This was the more extraordinary since the farmer gave the assurance that the seed was the same (King Edward) and from the same source, that the manurial treatment had been the same (namely a dressing of Fison's Mixed Artificial), moreover the soil type appeared to be identical on the two plots. The only difference lay in the setting of the "seed"; in the one case use had been made of a home-made dibbling machine while in the other the drill had been ploughed and the "seed" set in the ordinary way. In the former case presumably the "seed" had been set nearer the surface than in the latter and this would seem to have given rise to conditions favourable to the action of bacteria. As this suggests a possible method of control in bacterial diseases experiments will be made during this summer in order to test the efficacy of deep planting in

checking "Blackleg." Some of the tubers from the diseased plot in question have been obtained for this purpose.

ISOLATION OF THE ORGANISM CAUSING THE DISEASE.

In view of the fact that the material was collected rather late in the year the separation of the parásite from the great number of accompanying saprophytes has proved very difficult and tedious. Many attempts at isolation by the method of "poured plates" have given only disappointing results; five out of thirty of the original cultures of mixed organisms produced on potato slices vigorous rotting which could be carried on from potato to potato, but whenever attempts were made to obtain the active organism on plates of bouillon-gelatine or bouillon-agar the colonies that developed invariably proved to be those of organisms which had no power to produce rotting[1].

Successful isolation was finally obtained as follows: from one of the original cultures on potato-mush-agar a water suspension of the mixture was made and the cut end of a shoot of potato was immersed in it. After four days the stem showed characteristic blackening, and rotting of the pith. This rotten pith was then inserted in a hole made by a half centimetre cork-borer in a potato tuber which had been sterilised with mercuric chloride. After two days incubation at air temperature rotting was well established and from the margin of the rotted area a loopful was taken and inoculated upon potato-mush-agar. Attempts at isolation of the parasite from this culture by "poured plates" again proved abortive. A water suspension was made from the growth on potato-mush-agar and the cut end of a bean shoot (*Vicia faba*) was immersed in it, there being at this time no shoots of potato available. In four days the pith was well rotted to a height of five centimetres from the end. Poured plates in alkaline bouillon-agar were then made from the pith at the limit of disease. Several colonies appeared after three days and these were tested in turn as to their rotting power on a potato slice. Some thirty colonies were tried in this way with negative results, but three weeks after plating one colony was discovered which proved to be of a pathogenic kind. Exactly when this colony appeared it is impossible to say as it was quite old when tested and had ceased to grow. This proved to be a vigorous

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[1] Many of these colonies were produced by Micrococci and the regularity of their appearance suggests that they represent the organism described by Frank under the name *Mi rococcus phytophthorus* and this should be considered a *nomen nudum* until a pathogenic form answering to Frank's description shall be re-discovered.

·organism producing lively rotting of the potato. Whereas the impure cultures had always given from the first a pinkish-brown colour in the rotted tissue or a mixture of brown, black and yellow, the rot produced by the pure culture was not coloured at all until about a week after inoculation, when a slight pink tinge developed in the rotted part bordered by a brown stain in the surrounding tissue which finally turned almost jet black.

After two transfers to fresh sterile potato slices the organism was plated out on bouillon-gelatine and incubated at 19° C. On the second day thirteen colonies all apparently similar had made their appearance and had produced a basin-shaped liquefaction of the gelatine with a diameter of four to five millimetres. Each of the thirteen colonies was inoculated upon potato tissue and gave rise to the rot characteristic of the organism as described above. This was taken as proof of the purity of the culture and examination of the organism in stained preparations and under "dark-ground" illumination further verified this.

DESCRIPTION OF THE ORGANISM.

I. MORPHOLOGICAL CHARACTERS.

Form and Size. The organism is a short rod with rounded ends. When taken from the diseased tissue after 48 hours incubation at 20° C. the length of the single rod varies from $1·2\mu$ to $2·4\mu$ and the breadth from $0·7\mu$ to $0·8\mu$; pairs of organisms are very numerous and measure up to $3·5\mu$ in length. Taken from bouillon-agar the length of the rods is more variable and pairs of organisms are not of such frequent occurrence but the organism has a tendency to form in chains. The measurements were made upon preparations fixed five minutes in 4 per cent. formalin, stained 10 minutes in aqueous methyl violet and examined in oil.

Motility and Flagella. The organism is an actively motile bacillus and swims with a rapid rotation on its long axis. The flagella are peritrichous and three to six in number when taken from an agar slope, but many are uni-flagellate when taken directly from diseased tissue. Beautiful stained preparations of the flagella were obtained by a modification[1] of van Ermengen's method and also by Lowit's method.

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[1] This was a modification by Stevens and I am indebted to Dr R. S. Williams for having introduced it to my notice. Details of the method are given in Hewlett's *Bacteriology*, 5th Edition, 1914, page 115.

Staining. The organism stains well with the usual bacteriological stains. It exhibits no polar or other characteristic granules. It does not stain by Gram's method. Spores have not been observed.

II. CULTURAL CHARACTERS.

Stab Culture in Bouillon-gelatine. In acid gelatine (titrating + 10 of Fuller's scale), after 24 hrs. at 20° C., growth was visible to the bottom of the tube and liquefaction had commenced at the upper part of the stab. After 48 hrs. liquefaction was "infundibuliform" and had spread to about 8 mm. diameter at the surface. A granular deposit formed in the liquid gelatine and gradually sank to the bottom. The reaction of the liquid was strongly alkaline to litmus. In alkaline gelatine (− 1·5) growth was similar but rather more rapid.

Stab Culture in Bouillon-agar. Growth was granular and uniform throughout the length of the tube. The organism is obviously a facultative anaerobe. No difference in growth could be observed on media of acid or alkaline reaction.

Streak Culture on Bouillon-agar. After 24 hrs. at 20° C. the streak was 1·5 to 2 mm. wide with smooth margin; dirty white by reflected light; raised; "wet-shining." When collected in a mass on a platinum wire a faint yellow colour was perceptible.

Plate Culture on Bouillon-gelatine. After 48 hrs. at 20° C. the colonies had made their appearance and those on the surface had liquefied the gelatine; liquefaction basin-shaped; bluish-grey turbidity; granular deposit at bottom of depression.

Plate Culture on Bouillon-agar. Colonies visible after 48 hrs. at 20° C. Surface colonies dirty white; brown by transmitted light; round; "wet-shining"; raised ½ to 1 mm. above the surface; domed. Deep-lying colonies "punctiform."

Streak Culture on Potato-mush-agar. After 24 hrs. at 20° C. streak about 2 mm. wide and of a light chrome-yellow colour.

Streak Culture on cooked Potato. After 48 hrs. at 20° C. strong growth and chrome-yellow colour, surrounding tissue of a mouse-grey colour.

Bouillon + 5. Bouillon turbid after 24 hrs. at 20° C. No pellicle formed at the surface and no ring where the liquid meniscus came in contact with the glass. After four days a considerable deposit had formed at the bottom of the tube.

Potato Broth. Became turbid after 24 hrs. at 20° C. On the second day there was in one case a slight sign of a pellicle but this broke up

on shaking, sank to the bottom and did not re-form. In three other tubes there was not the slightest sign of pellicle formation. After eight days the liquid was neutral to litmus, and a small bubble of gas had collected in a Durham's tube.

III. Physiological Characters.

The culture used for these experiments was prepared as recommended by Harding and Morse(7) in their report of investigations on the physiological behaviour of various strains of soft-rot-producing organisms, successive transfers being made from bouillon to bouillon-gelatine and then to bouillon-agar, one night's incubation at 20° C. being allowed between each. A tube of Dunham's solution was inoculated copiously from the last and a loopful of this employed for each test. In all cases unless otherwise stated the temperature of incubation was 20° C.

10 *per cent. Witte Peptone* + 1 *per cent. Glucose.* Became slightly acid on the second day, on the fourth day it had become strongly acid and a small bubble of gas had collected in a Durham's tube. After fourteen days the gas occupied about 1/20 of the Durham's tube.

10 *per cent. Witte Peptone* + 1 *per cent. Lactose.* Acid appeared on the third day and gas on the fifth day. Final volume of gas as above.

10 *per cent. Witte Peptone* + 1 *per cent. Saccharose.* Acid and gas appeared on the fifth day. Volume of gas as above.

10 *per cent. Witte Peptone* + 1 *per cent. Mannite.* Acid and gas appeared on the third day. Volume of gas was about three times as large as in the cases of the other sugars.

Uschinsky's Solution. No acid and no gas produced. The litmus showed slight alkalinity and on the fourth day commenced to bleach from the bottom of the tube upwards. ·After fourteen days the liquid was entirely decolorised except for a depth of a few millimetres at the surface. The colour was partially restored on shaking.

Potato Broth + 2 *per cent. Glucose.* After eight days incubation at 25° C. reaction very slightly acid (practically neutral). Three minute bubbles had collected in a Durham's tube. No trace of pellicle formation.

Potato Broth + 2 *per cent. Saccharose.* After eight days at 25° C. reaction slightly acid. Bubble of gas less than 0·1 c.c. No sign of pellicle formation.

Nitrate Bouillon. Growth wa more vigorous than in bouillon

without nitrate. Nitrite was present after 24 hrs. and was still present after thirty days.

Diastatic Action. This was tested on a piece of potato standing in water. After fourteen days the liquid gave a true red colour with iodine, showing that all the starch had been hydrolysed. The diastatic action of the organism is fairly strong despite the fact that the starch grains appear to be unattacked in the cells of rotted potato tissue.

Milk. Coagulated on the sixth day. The curd was quite loose and was easily broken by gently tapping the tube. On the twentieth day the whey was well separated from the curd. The curd was still present thirty days after inoculation.

Litmus Milk. Became slightly acid on the third day, distinctly so on the fourth day; coagulation occurred on the ninth day, loose curd as above; the whey titrated on the thirtieth day was acid to the degree 0·06 N (+ 60 of Fuller's scale).

Dunham's Solution. Growth was not vigorous, only a slight turbidity was formed. When tested after fourteen days there was no sign of the presence of indol.

Thermal Death Point. 60 c.c. of potato broth was inoculated and incubated over night at 25° C. The turbid liquid was divided into six sterile tubes and these were subjected for ten minutes to temperatures ranging from 48° to 55° C. Inoculations made from these on potato-agar showed that the organism was killed at temperatures above 49° C.

INFECTION EXPERIMENTS.

Sterile Slices of living Potato. The potato was sterilised with mercuric chloride, washed three times with sterile water and cut into slices with a flamed knife. The slices were placed on wet blotting paper in sterile petri dishes. At 20° C. rotting was well established after 24 hrs., had penetrated to a depth of about 2 mm. and had spread to a radius of 1 cm. from the point of inoculation; at this temperature the whole of the tissue was decomposed. The rotted tissue had a strong alkaline reaction to litmus and gave off a volatile base which was identified by the sense of smell as trimethylamine; no other odour was perceptible. As stated earlier the rot was perfectly white and remained so in the incubator but on exposure to light a rose-pink colour developed. At air temperature 12° to 14° C. the rot usually progressed throughout the depth of the slice (5 to 6 mm.) but after spreading to a radius of 1 to 1·5 cm. from the point of infection it was frequently prevented from further advance by a resistant layer of cork; this cork formation was

accompanied by a brown stain in the tissue bordering the rotted area and finally the whole of the unattacked tissue assumed a dark brown to jet-black colour.

Sterile Slices of other Vegetables. At 20° C. vigorous rotting was produced in slices of carrot, white turnip, yellow turnip, celery, onion, Jerusalem artichoke, parsnip and sugar-beet. At air temperature these all rotted to a certain extent but much less rapidly than at 20° C., and rotting was frequently checked as in the case of the potato by a protective formation of cork.

Cut Shoots of Potato and Bean (Vicia faba). Shoots of these two plants placed in tubes containing 10 c.c. of a suspension of the organism in distilled water and kept in a green-house at 54° to 57° F., commenced to rot at the cut end on the second day. The rot accompanied by characteristic blackening of the stem steadily advanced upwards, so that after eight days a length of some four or five centimetres of the stem had been completely decomposed. Controls under the same conditions remained perfectly healthy for three weeks.

Prick Infection of the Stem of growing Plants. The plants used had been forced in pots during the winter in a green-house with a regulated temperature of 54° to 57° F. When the infections were made the plants inoculated had sturdy upright stems eight to ten millimetres in diameter and twenty to twenty-five centimetres in height, but leaf production was very meagre and some of the plants were showing signs of precocious flowering. They were not very robust specimens but served quite well for the purpose of this research. When infection was to be made below the level of the soil this was removed to a depth of about two inches and subsequently replaced. Before inoculation the stems were washed with alcohol and allowed to dry, puncture infections were then made with a stout platinum wire bearing the inoculating material, and the stems were bound closely with one centimetre strips of tin foil in order to protect the wounds against the entrance of soil and loss of moisture. In all, ten plants were inoculated in this way, four below and six above the level of the soil. In every case successful infection resulted while control plants remained perfectly healthy. On three occasions the organism was successfully re-isolated from such infected stems. One typical experiment may be cited in detail The stem of a plant bearing one shoot only, 25 cms. in height and 8 mm. in diameter, was inoculated as described one inch below the level of the soil with material from a rotted potato slice. This culture had been incubated over night at 20° C. and represented the eighth transfer from the original,

and the third transfer from the isolation of the organism in a state of
purity.   The first sign of rotting of the stem was observed on the tenth
day after inoculation; on the thirteenth day the stem was black to a
height of two centimetres above the level of the soil; it was then pulled
up and found to show the signs characteristic of the disease, intense
blackening of the epidermis, dark brown stain in the vascular bundles
and complete destruction of the pith at the base of the stem.   The
epidermis was seared along one side with a hot knife and cut longitudi-
nally, the sides were laid back with sterile forceps and a portion of the
most recently attacked pith was transferred to a tube of sterile water.
At the same time a portion of the pith was placed upon a slice of potato
and after 24 hours at 20° C. had produced a white rot some 4 mm. in
depth and of 1 cm. radius.   Dilution plates in bouillon-gelatine were
made from the water suspension of the diseased pith and after 48 hours
incubation fifty colonies had developed on the first plate, these were all
similar in appearance and had produced liquefaction, as described
earlier, varying from 1--3 mm. in diameter.   A loopful from each of ten
of these colonies was "spotted" on a slice of sterile living potato and
in each case characteristic rotting of the tissue resulted, thus the four
rules of Koch for the establishment of the pathogenicity of the organism
were fully complied with.

COMPARISON OF THE ORGANISM WITH PREVIOUSLY DESCRIBED PRODUCERS OF "BLACKLEG."

The organism here studied differs from *B. caulivorus* or organisms of
the fluorescent type in not producing a green fluorescence in artificial
culture and from *B. solanacearum* and *B. solanincola* in its power
to liquefy gelatine, a character which these two organisms do not
possess.   It is also clearly different from an organism which has been
described by Kramer[11] as causing a rot of the tuber in that it does not
produce gas and butyric acid when cultivated on potato tissue and in
certain nutrient solutions.   There remain to be considered *B. atro-
septicus*, *B. solanisaprus*, *B. melanogenes* and *B. phytophthorus*.   The
first three of these as stated earlier (page 484), have been shown to be
strains of one and the same species by the fact that under certain
cultural treatment the physiological differences upon which separation
of these was based fail to appear.   The description which Morse[22]
gives of the characters of these organisms after they had been cultivated
through several changes of potato-broth agrees almost entirely with
the description given in this paper and with the original description of

Pethybridge and Murphy (15) for their species *B. melanogenes*. It is thus clear that in all these cases the same organism is being dealt with and in agreement with the opinion of Morse the proper designation of the organism is *B. atrosepticus*. Since several strains of this organism have been isolated in different localities it is of interest to note that the one found in Lancashire is the same as that previously found in Ireland.

It is to be regretted that Morse was unable to obtain a virulent culture of *B. phytophthorus* since it has been shown by Pethybridge and Murphy (15) that this species differs only very slightly from their organism, and it seems probable that a comparison of this organism along with the others studied by Morse would have proved it to be identical with them. However, being unable to obtain a trustworthy culture of *B. phytophthorus*, and in deference to the opinion of Smith(18) that this species is not identical with *B. solanisaprus*, Morse was forced to exclude it from the species *B. atrosepticus*. Since the statement by Smith was in all probability based upon physiological differences which have been shown by Morse to be inconstant a comparison of the cultural characters of *B. phytophthorus* and *B. solanisaprus* on the lines laid down by Morse is highly desirable.

SUMMARY.

The organism which produces "Blackleg" of the potato in Lancashire is *B. atrosepticus* (van Hall).

It is in all respects identical with the organism responsible for this disease in Ireland and described by Pethybridge and Murphy under the name *B. melanogenes*.

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THE CHANGES TAKING PLACE DURING THE STORAGE OF FARMYARD MANURE.

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CONTENTS.

INTRODUCTION.

THIS investigation began in an attempt to account for the loss of nitrogen that occurs during the cultivation of land rich in organic matter or liberally supplied with farmyard manure. For example, the Rothamsted plots receiving annual dressings of farmyard manure return in the crop only about one-third of the added nitrogen; no great quantity is stored in the soil, and there is little apparent drainage water, although no doubt some seepage occurs. Altogether little more than one-half of the nitrogen can be accounted for, and it is difficult to avoid the conclusion that some escapes in the gaseous state, being liberated as the result of biochemical processes in the soil. Similar results have been obtained for prairie soils: the amounts of nitrogen lost from the soil are much greater than those recovered in the crop.

Direct experiments with soil are in progress in our laboratories; but it will readily be understood that an amount of nitrogen which is of

great importance in crop production when reckoned over an acre of land, is, nevertheless, very small on the few grams of soil used in a laboratory experiment lasting only for a few weeks.

The difficulties arising out of the smallness of the amount to be measured led us to seek out a parallel case where the absolute quantities are larger, and where, therefore, there is greater hope of getting precise information. The manure heap affords such a case: it is the seat of biochemical decompositions which in several important respects resemble those in the soil: in particular it undergoes loss of nitrogen, but the amount is far greater than that suffered by soil, and the loss proceeds much more rapidly. The elucidation of the causes of loss of nitrogen from a manure heap appeared by no means a hopeless task, and would, if successful, afford a useful guiding hypothesis for starting an investigation on the loss of nitrogen from soil.

As the work progressed, however, the technical importance of the problem led us to widen the scope of the investigation, and to make it deal with the changes in the manure heap independently of their bearing on the changes within the soil.

When a manure heap is put up fermentation rapidly sets in accompanied by a considerable rise in temperature. All the organic constituents of the heap appear to alter: we have devoted most attention to the nitrogen compounds, and this course is justified by the field experiments, but the non-nitrogenous constituents also change to an equal, and sometimes to a greater extent. As a rule the loss of nitrogen proceeds *pari passu* with that of the other constituents, so that the percentage shows no great change or only a small falling off: under certain circumstances, however, it proceeds more slowly, leading to an actual increase in percentage amount, though of course a decrease in absolute quantity.

The loss of nitrogen seems to fall on all the nitrogen compounds excepting perhaps the amides. The ammonia invariably falls off in amount: in no case have we observed an increase. The amides usually remain constant, sometimes they fall but they do not increase. The more complex nitrogen compounds,—obtained by difference,—usually fall, but in some cases they remain constant. But there is nothing to show what reactions have taken place, or how the losses have occurred.

In laboratory work on bacterial decomposition of protein there is always an accumulation of ammonia, so also there is in the decompositions taking place during sewage purification. In these cases the reaction appears simply to be the ordinary hydrolysis of protein to

amino-acids, followed by a splitting off of ammonia from the amino-acids. But in the manure heaps, as already stated, ammonia does not accumulate, and there is nothing to show whether the decomposition proceeds in the same manner as in these cases, or on wholly different lines.

As a manure heap is rather a cumbersome subject the experiments designed to elucidate this point were made in the laboratory under conditions as nearly as possible similar to those obtaining in the heap. It was found that the decomposition proceeded normally, and ammonia accumulated just as in putrefaction, in sewage decompositions, etc.

It appears, therefore, that the decomposition of nitrogen compounds in manure follows the ordinary course, giving rise to ammonia, with amino-acids presumably as the intermediate product, but that the decomposition in the heap is masked by two actions: the non-accumula-tion of ammonia, and the loss of nitrogen.

Experiments showed that ammonia might be lost by direct volatilisation, by conversion under certain definite conditions into nitrate, or to a small extent by reconversion into complex nitrogen compounds. Of these the two former are known to proceed in sewage decompositions, but the latter has, so far as we know, only been studied in connection with the manure heap.

The loss of nitrogen cannot be wholly explained by the volatilisation of ammonia. Under laboratory conditions only little volatilisation takes place, and in any case the ammonia is caught in acid traps. Yet there still remains a substantial loss,—15 %, or more, of the total nitrogen,—which cannot be accounted for. We have been able to show that there is an evolution of gaseous nitrogen during the decomposition, which thus completes the account of the loss. This loss does not go on under anaerobic conditions; the complex compounds break down, yielding an equivalent amount of ammonia. A similar result has been obtained in sewage decompositions. The loss, therefore, is not caused by a simple reduction of some compound in the manure; e.g. it is not a simple denitrification.

Nor does it go on under wholly aerobic conditions; here again the yield of ammonia *plus* nitrate is quantitatively equivalent to that of the complex nitrogen compounds broken down. Even when nitrification proceeded vigorously there was still no loss of nitrogen. The loss, therefore, does not arise from a simple oxidation.

The loss goes on only under the mixed aerobic and anaerobic con-ditions which arise when actual manure, trampled, mixed and compacted

by beasts, is exposed to air. It appears, therefore, to be a complex process, requiring both aerobic and anaerobic conditions. It is shown that the loss can be explained on the following general hypothesis.

Some of the molecular groupings which arise under anaerobic conditions are known to be unstable under aerobic conditions, and *vice versa*. Thus, one of the derivatives of propionic acid formed under anaerobic bacterial decomposition of protein shortens itself and becomes a derivative of acetic acid as soon as air is admitted.

Most of the cases on record are eliminations affecting the number of carbon atoms, but it seems quite conceivable that nitrogen should be eliminated in similar manner.

On this view we suppose air to diffuse into the heap and to give rise to CO_2, so that the atmosphere in the heap is a constantly varying mixture of oxygen, carbon dioxide, and nitrogen. When the oxygen happens to be in sufficient excess the aerobic processes go on; when, on the other hand, the oxygen falls too low the anaerobic changes set in, and some of the compounds formed under the aerobic conditions become unstable, and lose their nitrogen. Conversely, products formed under anaerobic conditions may lose nitrogen as soon as more air diffuses in.

Alternate nitrification and denitrification is a special case of this hypothesis: nitrate may be supposed to form during the aerobic conditions, and then become denitrified under the anaerobic conditions. We have shown that this actually takes place to some extent: nitrates are formed on the outside of the heap wherever there is sufficient dryness and sufficient air, but they decompose directly they are washed into the interior. We do not suppose that this particular process accounts for the whole of the loss: there are mechanical difficulties in the transfer of sufficient quantities of nitrate from the outside to the inside of the heap; it is possible of course that nitrates are formed in the interior also but that they decompose at once so that they cannot be detected. We have not in the present paper attempted to pursue this matter more closely.

We now come to the application of our results to the storage of the manure heaps.

Field experiments show that the best results are obtained when the manure loses as little dry matter as possible, and when it contains the maximum of total nitrogen and ammonia.

Laboratory experiments show that these conditions are attained when the manure is stored under *anaerobic* conditions at about 26° C.

Experiments with manure heaps show that results similar to those of the laboratory are not obtained in manure heaps, but that on the contrary both nitrogen and ammonia are lost. Improvements can be made by compacting and by sheltering the heap, even a little shelter is good. But at best the heap is an imperfect method, and probably in the best practice it has been developed about as far as it can go.

Our results indicate that the main hope for further improvement lies in storing manure in water-tight pits or tanks so arranged as to allow of the attainment of anaerobic conditions and suitable temperature. Whether the improvement would be profitable could only be settled by trial; it paid in the case of silage, however, and the silo made of reinforced concrete has displaced the old stack silo because it was found more profitable

The Hon Rupert Guinness has kindly given us facilities for carrying out the necessary large scale trials on his farm at Hoebridge

HISTORICAL

In order to see the problem as a whole it is necessary to go somewhat into the history of the art and science of managing the manure heap. We shall not do this in any detail, although in point of fact an exceedingly interesting account might be written showing how, at every stage, the best practice has been in accordance with current scientific ideas, while ordinary practice has lagged behind for want of means to carry out what was known to be best

The management of the manure heap had already reached an advanced stage of development in Roman times Varro[1] writing about 40 B C insists upon two points, first, that the manure should be rotted before use and therefore there should be two heaps,—one fresh, and one rotted, secondly, that the heap should be kept moist by allowing water to run into it and also by protecting its sides from the sun by twigs and leaves, "for the sun must not suck out beforehand the goodness which the earth requires " · Columella, about 90 A D., as usual amplifies Varro, and gives details for constructing the place or pit where the manure is to be kept[2], and for turning the manure in summer time to facilitate rotting: this rotted manure was needed for the corn, while the stronger fresh manure was used for grass He suggested

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[1] Varro, *Rerum Rusticarum*, lib I cap 13

[2] Columella, *Rerum Rusticarum*, lib II cap 15: "With bottom shelving in the manner of ponds, well built and paved, that the moisture shall not pass through, for it is of great importance that the dung retain its strength by the juice of it not being dried up."

that the manure from different kinds of beasts should be kept separately, but if corn only was being grown there was no necessity for this. Thus, three guiding rules were laid down:

1. The manure must not be allowed to become dry,
2. It must be rotted before being applied to corn,
3. If possible the different kinds of manure must be kept separately.

Succeeding writers—Cassianus Bassus who compiled the *Geoponica* about the middle of the tenth century, Crescentius in the thirteenth century, and the writers of the Renaissance period—generally transcribed Varro and Columella without always quite understanding them, and added so little that the three rules just given were almost universally quoted right up to the experimental times of the eighteenth century.

Neither Varro nor Columella had anything to say about the bad effect of rain on the manure heap: their whole concern was to prevent it from getting too dry. No doubt in Italy the heap was more likely to suffer from sun than from rain. In England and North Europe the case was different, but as the agricultural teaching was derived from the Latin writers no account seems to have been taken of this difference, and we can find no particular recommendation to guard against rain. It is impossible to estimate how much was lost to mediaeval man by the strict adherence to the instructions of Columella in spite of the difference in conditions between Roman and North European husbandry.

Mortimer[1], one of the most polished writers of his day, recognised that rain was harmful but he was much too good a student of Columella to leave out the instructions with regard to the making of the pit. "The common way (of making a manure heap) is by laying of the Dung in heaps till it rots; but the way that would be most profitable to the Husbandmen is to make near his House or Barns a large pit...and to pave it with Stone or Chalk that it may detain the moisture of the Dung...." Then he breaks away from the classical instruction and adds—"but if you can have a covering over it so as to keep all the Rain-water out of it...it will much improve it: For the Rain-water that runs from it, carries away the Salt of the Dung with it, which is the chief cause of its fertility. But if you cannot have such a conveniency, lay it as thick on heaps as you can, and in the moistest lowest places you have, covering the top of it with Turf or other Earth to prevent the Sun or Wind from extracting and drying the virtue of it:...for the better and greater the quantity of your Dung is, the better will be your .

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[1] *The Whole Art of Husbandry,* by J. M. Esq., F.R.S., 1707, p. 96   In later editions the author gives his full name.

Crop: and the increase of your Crop will make an increase of your Dung..." etc.

But these instructions were not generally followed, and so it happens that William Ellis, the farmer writer of Little Gaddesden, Herts, was driven in 1731[1] to condemn utterly and vigorously the common plan of throwing out all dung in separate little heaps in the farmyard, and leaving it there exposed to the wash of the rain. All these, he says, should be mixed together and kept under cover to protect them from the weather, or failing a suitable covered place, "then as the Beast Dungs are made they should be lain in one great Heap or Dung Hill, which, next to Cover, will preserve their good Properties in a great Measure from the Power of Rains and Droughts; and, as the black Water drains from it, it ought to be carefully preserved, by causing it to run into such a Receptacle or Reservoir, as will give the Farmer an Opportunity to carry it out in a Tub or Barrel, for throwing it over the Dunghill, or to scatter it over Plowed or Grass land."

Later writers, Donaldson[2] and others add little to this.

In the Experimental Period at the end of the eighteenth century the first point to be attacked was the question of rotting the manure So long as it was applied to wheat in the large quantities common at the time[3] some preliminary rotting was needed to kill the weeds, but with the introduction of hoed or fallow crops this procedure became unnecessary. A number of farmers pointed out in the *Annals of Agriculture* that the fresh dung drawn straight from the beasts and applied to these cultivated crops gave better results than rotted dung[4].

The ancient rule about the need for rotting the dung, therefore, was seen to be without foundation, and this was confirmed on the chemical side by Gazzeri[5], and finally by the detailed analyses of A. Voelcker[6] in 1856. It is now generally admitted that the making of the manure heap is a matter of convenience only, and not of necessity, and that the most economical plan is simply to leave the manure under the beasts until it is wanted,- provided always that the yard is covered.

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[1] Wm Ellis, *The Modern Husbandman*, for the month of November, pp. 67 et seq in the 1743 edition

[2] James Donaldson, *Modern Agriculture, or the present state of husbandry in Great Britain*, 1796, pp. 26, 245 et seq.

[3] Eighteen to twenty-four cartloads, each containing 16 to 18 cwt, per statute acre, according to Donaldson.

[4] These papers are summarised by Young in his interesting "Essay on Manure," *Bath Soc. Papers*, vol. x. 1804   See also Young's *Farmer's Calendar*, Art Manure

[5] Quoted in Boussingault, *Economie Rurale*, 2nd edition, 1851, vol. I. p. 707.

[6] A. Voelcker, *Journ. Roy. Agric. Soc.* 1856, **17**, 191–260.

33—2

The second notable advance was made about 1800 by von Thaer[1], the most distinguished agricultural chemist of his day. It is true that he was still adopting Columella's recommendations already given; but he made some experiments,—only on a very small scale, using a few ounces of manure, and with the old analytical methods of dry distillation,— which, he considered, showed the necessity of excluding air from the heap: he supported, therefore, the practice known, but not common, of sending bullocks or carts over the heap to compress it, and then covering it with a layer of earth. Boussingault[2], who took many of his ideas from von Thaer, adopts this one *in toto*; but he also re-examined the Columella recommendations that von Thaer had accepted, and promptly dismisses the idea that various manures should be kept separate. But Boussingault's great achievement was to reveal the true nature of manure by demonstrating the connection between food and dung, and he showed that the composition of the dung was simply that of the litter ⊢ the food, *less* anything retained or breathed out by the animal. Thus, when the necessary physiological data were available, the composition could be calculated by simple arithmetic. This constitutes the greatest advance of all for it afforded the basis on which all modern work has since developed.

We may now briefly summarise the results of the 50 years' work which began with Young and ended with Boussingault:

1.   Farmyard manure represents the litter ⊢ that portion of the food which the animal does not retain or breathe out; its composition is therefore directly related to that of the food (Boussingault, 1840).

2.   The manure gives the best results when it is used in its unaltered state and without any preliminary rotting: the manure heap is, therefore, only a matter of convenience and not of necessity (Young).

3.   If a heap has to be made it must be done so as to exclude as much air as possible (von Thaer, 1800).

4.   It must be protected from rain: if no cover is available a layer of earth affords useful protection (Mortimer, 1707, but may be older).

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[1] A. von Thaer, *Grundsatze der rationellen Landwirtschaft*, 1810, Bd. 2, pp. 87 et seq. The experiments are described in a paper by Thaer and Einhof entitled "Versuche und Beobachtungen uber die Excremente vom Hornvieh, und ihre Faulniss," published in Hermbstadt's *Archiv der Agriculturchemie*, 1804, **1**, 255–304. Nothing illustrates more vividly the enormous strides made by agricultural chemistry during the period 1804 to 1840 than a comparison between this and Boussingault's papers.

[2] *Economie Rurale.* Boussingault thought that some of the nitrogen was actually exhaled: it was not till physiologists had proved this not to be the case that the full value of his work was appreciated.

5. It must not be allowed to become dry (noted by Varro, very old).

6. In practice the best way of securing all these conditions is to leave the manure under the animals, kept in a covered yard or box, until it is wanted for the field, and then to draw it out and apply it without delay.

The application of these results is obviously easy in the case of bullocks tied up to be fatted, and in some cases the old deep stall was revived for holding the bullocks,—an adaptation of the plan used in Germany from mediaeval times, but discarded on the introduction of the succulent fodder crops because the volume of dung and urine became too great to be manageable in the stall as originally made.

No advance of any consequence has been made in the best practice since these times; there has, however, been considerable advance on the scientific side.

Boussingault's important generalisation as to the relation of manure to food has formed the basis of most of the work. Physiologists proved that no nitrogen was exhaled by the animal, and Lawes and Gilbert's analyses showed how much was "fixed" in the flesh, it was, therefore, a simple matter to determine how much of the nitrogen of the food ought to be in the manure. Further, the digestibility methods of the physiologists made it possible to ascertain the distribution of the various fertilising constituents,—the nitrogen, phosphoric acid, potash, etc.,—of the food between the urine and the faeces, and experiment soon showed that the material digested and excreted in the urine had a higher fertilising value than the undigested material of the faeces.

The first application of these principles to the manure heap was made by Lawes and Gilbert from 1854 onwards[1], and had to do with the residual manurial value of various feeding stuffs. Henneberg at Weende[2] followed up the work and it was developed in Germany by Maercker (who began at Weende) and Schneidewind[3] in 1898. These investigations showed that the nitrogen actually present in the manure was never equal to the calculated amount; there was always a loss, even when precautions were taken to prevent loss by drainage. The German Agricultural Society realised the practical significance of this result, and in 1896 urged the Agricultural

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[1] Their last paper on the subject summarises their work and that of the other investigators up to that time. *Journ. Roy. Agri Soc.* 1895, pp. 47–146..

[2] *Beiträge zur Begründung einer rationellen Fütterung der Wiederkauer,* Heft 1, 1860.

[3] Maercker and Schneidewind, *Landw. Jahrbücher,* 1898, **27**, 215–40

Experimental Stations to take the matter up, and find out the cause of the loss and if possible some means of preventing it.

These experiments are discussed later on: the general conclusion as to the cause of the loss was that it might arise from the evolution either of ammonia or of gaseous nitrogen. Numerous experiments by many workers showed that the evolution of ammonia was only slight except when the heap was actually hot and giving off vapours: usually the outside layers of the heap retained it. The loss was therefore attributed mainly to gaseous nitrogen.

The efforts to stop the loss met with no success. Schattenmann[1], a large horsekeeper in Bauchsweiler, Alsace, managed enormous heaps of horse manure extremely well and he added either sulphate of iron or gypsum. The idea gradually developed that gypsum "fixed" the ammonia and prevented loss. As this seemed promising Joulie[2] tried a number of likely "fixers," but found them ineffective. Similar negative results were obtained by Aeby and others[3], and by Pfeiffer[4] in the experiments organised by the German Agricultural Society. There is no evidence that any "fixer" is of any use.

Another application of Boussingault's principle led to results which are probably considerably exaggerated. Maercker and Schneidewind[5] found that the amount of quick acting nitrogen in the manure (i.e. ammonia and amide), is considerably less than the digestible nitrogen in the food, while the nitrogen in the form of complex nitrogen compounds is considerably more than corresponds with the indigestible nitrogen in the food. Later investigations have shown that some conversion of ammonia into complex nitrogen compounds may take place, though it is doubtful whether in normal circumstances it attains the high value given by Maercker and Schneidewind.

The changes observed in the manure heap.

We shall begin the account of our investigations by describing the changes that we actually found in manure heaps stored under known conditions, and shall then proceed to deal with the experiments designed to elucidate the reactions by which they are produced. As the changes

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[1] Schattenmann, *Annales Chim. Phys.* Series 3, vol. IV. p. 358. The work is also quoted in Boussingault's *Economie Rurale*, 2nd edition, vol. I. p. 701.

[2] *Annales Agronomiques*, 1884, **10**, 289–301.

[3] *Landw. Versuchs-Stat.* 1897, **48**, 247–360.

[4] *Landw. Versuchs-Stat.* 1897, **48**, 189–245.

[5] *Landw. Jahrbücher*, 1898, p. 215.

are extremely complex we have not attempted to follow them completely, and we are justified in this course by the field experiments which we shall now describe.

The relationship between the composition and the crop producing value of farmyard manure.

Farmyard manure has to serve two functions in the soil: to supply nitrogen in various forms, and also to supply organic matter which has certain well-known physical effects on the soil. It is not difficult to estimate the nitrogen in some of its combinations, but it is difficult to give any analytical figures except the total dry matter that will show any relation to the physical effects. In order to discover how far the analytical results were related to crop producing power our experimental manure heaps were applied to the land at the conclusion of each experiment, and the resulting crops were weighed.

The experiments were only continued for one season: our previous experience indicating that the later seasons bring out no new differences between different samples of farmyard manure, and indeed only tend to obliterate the old ones.

The results obtained from dressings of various samples of farmyard manure are given in Table I.

TABLE I.   *Relation between crop producing power and chemical composition of samples of farmyard manure.*

1915.   Potato Experiments, Rothamsted.   10 tons per acre of stored farmyard manure applied.

	Control	1	2	3	4
Crop yield, tons per acre	5·11	9·00	8·82	8·02	7·38
Percentage increase ...	--	76	73	57	44
History of manure					
Bullock dung stored for 3 months		Compact under cover	Loose under cover	Loose in open	Compact in open
Percentage composition of manure					
Dry matter	—	22·2	23·4	18·4	16·5
Total nitrogen ...	—	0·523	0·567	0·437	0·381
Nitrogen as NH$_3$ ...	--	0·063	0·035	0·012	0·012
„ „ amide ...	—	0·051	0·054	0·048	0·038
„ „ other compounds	—	0·409	0·567	0·377	0·331

1916. Wheat Experiment, Woking.   6·0 tons No. 1 and 6·2 tons No. 2, applied per acre, representing in each case 10 tons per acre of original manure.

	Control	1	2
Corn yield (total produce) lbs. per acre ...	3280	4056	3756
Percentage increase	—	24	15
History of manure			
Mixed dung, stored 5 months	.	Trampled heap, thatched	Trampled heap in the open
Percentage composition of manure.			
Dry matter	—	29·7	29·6
Total nitrogen		0·819	0·759
Nitrogen as NH₄	—	0·119	0·086
„ „ amide		0·047	0·043
„ „ other compounds	—	0·653	0·630
Weight of manure applied, tons per acre	—	6·0	6·2

1916.   Wheat Experiment, Rothamsted. · 10 tons per acre of stored manure applied.

	Control	1	2	3	4
Crop yield (total produce) lbs. per acre . .	4943	5792	5649	5364	5185
Percentage increase ..		17	14	9	5
History of manure					
Bullock dung stored for 9 months		Compact under cover	Loose under cover	Compact in the open	Loose in the open
Percentage composition of manure.					
Dry matter	—	21·4	26 0	19 5	24·5
Total nitrogen .	—	0 523	0 674	0 474	0 580
Nitrogen as NH₃		0 040	0 015	0 007	0·012
„ „ amide ..		0·044	0 058	0 039	0·051
„ „ other compounds ...	—	0 439	0·601	0·428	0·517

1916.   Potato Experiments, Rothamsted.   16·1 tons of No. 1, 14·3 of No. 2 and 15·9 of No. 3 applied, representing in each case 20 tons per acre of original manure.

	Control	1	2	3
Crop yield, tons per acre .	2·63	4·00	3·91	3·65
Percentage increase ...	—	52	48	39
History of manure.				
Cow dung stored for 5 months		Compact under cover	Compact, covered with soil in the open	Compact in the open
Percentage composition of manure.				
Dry matter	–	20·2	20·6	20·9
Total nitrogen	—	0·371	0·391	0·382
Nitrogen as NH₃ ...	-	0·008	0·011	0·010
„ „ amide ...	—	0 037	0·027	0·024
„ „ other compounds...	—	0·336	0·353	0·348
Weight of manure applied, tons per acre	—	16·1	14·3	15·9

In the 1915 potato experiments, samples Nos. 1 and 2 are close together in crop producing power: No. 1 appears the better but the difference is within the limit of error. On analysis it is poorer than No. 2 in dry matter, total nitrogen and complex nitrogen compounds, but it is distinctly richer in ammonia. No 3 is considerably poorer: it contains less of everything especially ammonia. No. 4 is the poorest of the lot, and it also comes out lowest on analysis.

In the 1916 wheat experiments at Woking the same general relationships come out, the total nitrogen and ammonia are both higher in sample 1 than in sample 2 and the crop producing power is also higher—more, indeed, than the figures indicate because through a misunderstanding a furrow was run down the centre of the plot receiving sample 1, so that the yield was depressed over that portion of the area: no allowance has been made for this.

The 1916 wheat experiments at Rothamsted on the whole fit in with the others: No. 1 has by far the highest ammonia content and comes out best in spite of the fact that its total nitrogen is lower than in 2 or 4. No. 2 follows close in crop producing power, its high total nitrogen making up for its lower ammonia content: No. 3 is far behind in crop producing power and in nitrogen and ammonia. The position of No. 4 is not explained by the analyses: it was a loose heap stored in the open with less crop producing power than the figures indicated.

In the potato experiments also the manure stored in the open is of less fertilising value than the figures indicate, and on the other hand, the sheltered heap No. 1 has in comparison with the exposed heap No. 3 some value which the analysis does not show.

Our general conclusion is that none of the analytical figures by themselves would indicate the crop producing value with absolute certainty, but a consideration of the total nitrogen and of the ammonia together, especially if the ammonia is weighted, gives a result which is in accordance with the crop returns. Exceptions arise when sheltered heaps are compared with exposed heaps; the latter have less crop producing value than the analysis indicates.

Berry's investigations[1] of manure heaps in the South-West of Scotland and the Leeds experiments[2] both show a connection between composition and crop producing power. The results are:

[1] *West of Scot. Bull.* No. XIV. 1914.   [2] *Leeds Bull.* No. 90, p 9

Berry, West of Scotland.

				Percentages increases	
Potatoes ...	...	...	...	57	49
Turnips ...	..	...	..	45	38

History of manure.	Compact under cover	Compact in open
Cow manure stored		

Percentage composition.					
Dry matter	...	...	. .	21·4	19·15
Total nitrogen	...	...	...	·406	·375
Nitrogen as NH_3		...	...	·023	·021

Garforth, Leeds.

	Total yield, 1910 and 1911, cwts per acre	
Hay .	81·5	77

History of manure	Liberal concentrated food	Moderate concentrated food
Bullock manure		

Percentage composition			
Dry matter .	.	21·1	18 7
Total nitrogen ..		·63	·44
Nitrogen as NH_3	.	·19	·08

The changes taking place in the manure heap.

Directly a manure heap is put up the temperature begins to rise. Typical curves are shown in Fig. 1: their general shape is the same in all cases, there being first a rapid rise to a maximum and then a falling off. The extent of the rise depends on:

(*a*) the nature of the manure,—horse manure attaining a higher temperature than bullock manure, which in turn becomes hotter than cow manure;

(*b*) the degree of compacting,—the compact heap never becoming as hot as the looser heap in the initial stages;

(*c*) whether the manure is stored under cover or in the open,—heaps under cover rising to a higher temperature than those in the open;

(*d*) whether the heap is left undisturbed or is turned. On remaking the heap there is a considerable rise of temperature.

The fluctuations when the heaps are stored in the open are affected by the rainfall, a period of heavy rainfall being followed by a lowering of temperature, and a period of drought by a rise:
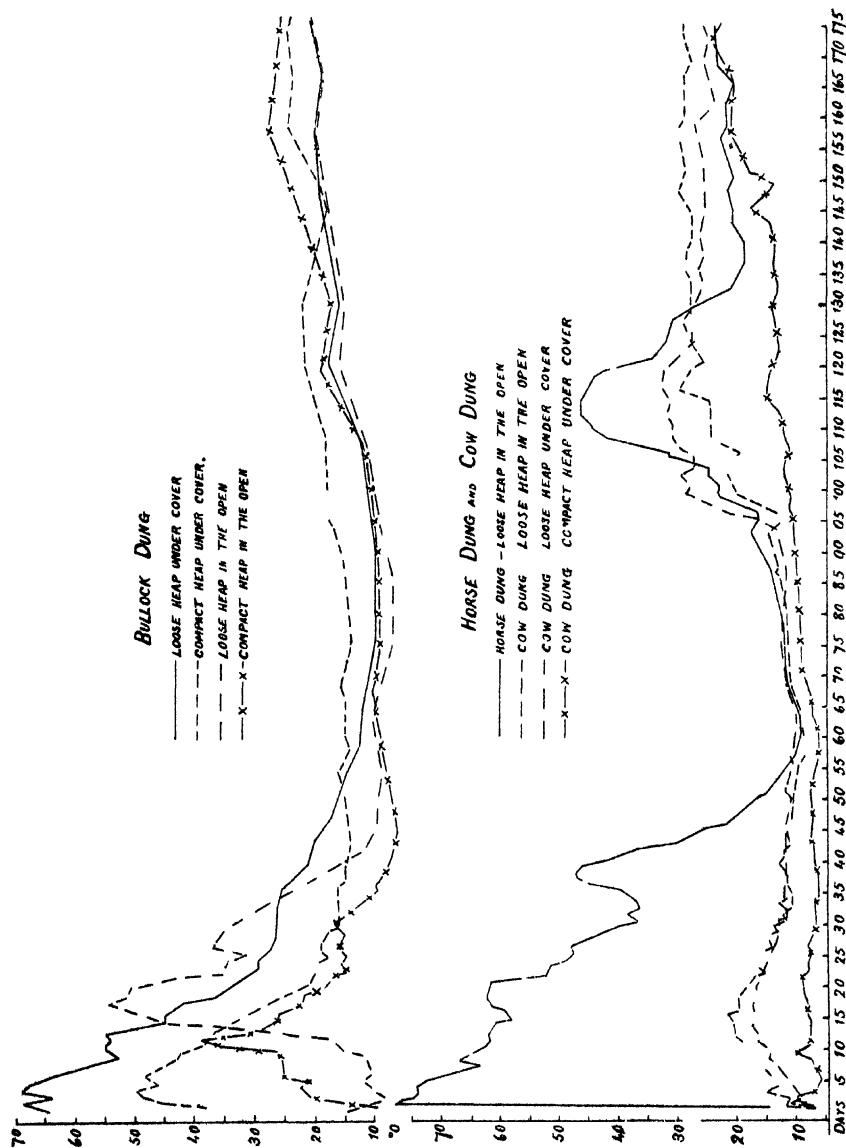
Fig 1   Changes in temperature of manure heaps stored under various conditions.   The heaps of horse and cow manure (lower part of diagram) were re made after the 90th day.

The effect of compacting is shown in the following table of maximum temperatures attained in the various heaps:

	Stored for 3 months under cover		Heaps re-made and then stored for a further 3 months		Stored for 3 months in open	
	Compact	Loose	Compact covered	Loose covered	Compact	Loose
Cow manure,						
Jan. 23—April 30, 1914 ...	9°	16°	22°	32°	—	21°
Bullock manure,						
Jan. 7—April 14, 1915 ...	51°	71°	—	—	40°.	55°

This difference in heat production obviously involves a considerable difference in the amount of dry matter decomposed, the loose heaps suffering greater loss than the compact ones. The percentage losses of dry matter were:

	Stored for 3 months under cover		Re-made and then left stored for a further 3 months		Stored for 3 months in open	
	Compact	Loose	Compact	Loose	Compact	Loose
Cow manure,						
Jan – April, 1914	4	7	9	26	—	21
Bullock manure,						
Jan. April, 1915	30	35	—	—	39	41

The losses in dry matter from heaps stored under similar conditions show some connection with the rise in temperature. But there is a difference between the exposed and sheltered heaps: the exposed heaps lose more dry matter than the covered heaps, and do not attain so high a temperature. The smallest change takes place in the heaps stored compact and under cover, and the greatest in heaps loose and exposed to the weather.

The dry matter of the heap is very complex in composition, and we have not attempted to follow the changes in all its constituents; we have confined ourselves to the nitrogen compounds because of their obvious importance. Further, we have not gone into the question of condition of the manure. We fully recognise its importance, but methods of investigation are lacking and for the present the safest plan is to accumulate observations. It has been noticed by Voelcker[1] that the addition of hay to a ration of straw, roots and cake much improved the condition of the dung. Further observations in this direction are very desirable.

[1] *Journ Roy Ag. Soc.* 1913, **74**, 410

The details of the analyses are given in Table IX, but to facilitate discussion some of them are given in diagram form in Figs. 2—5.

Compact heaps under cover. We begin with this as the simplest case. The most complete degree of compactness was attained in a heap of cow manure where there was sufficient faecal matter to allow the heap to be plastered down and made thoroughly solid. The temperature remained throughout sufficiently low to prevent serious evaporation. The heap was then left for the period Jan. to April, 1914 : various changes took place which may be summarised as follows, the initial quantities in each case being put at 100 :

	At start	After 3 months	Loss
Total dry matter	100	95·6	4·4
Total nitrogen	100	100	Nil
Including nitrogen as NH_3 ..	19	15	4
„ „ amide ...	10	9	1
„ „ other compounds	71	76	+ 5*

Maximum temperature attained 9° C.

* + indicates a gain in this and subsequent tables.

The results are set out in diagram form in Fig. 2.

During the period of storage there was a considerable evolution of gas,—mainly of carbon dioxide, marsh gas and hydrogen. A sample drawn from the middle of the heap has the following percentage composition :

CO_2	Combustible gas (CH_4, H_2, etc.)	N_2	O_2
73	26	1	Nil

The nitrogen compounds underwent certain transformations but suffered no loss in total nitrogen : this is an extremely important point which will be more fully discussed later on. The ammonia decreased in amount, but the "other compounds" showed a corresponding increase, indicating a conversion of ammonia into more complex substances. This particular change is not common in cow or bullock manure, and we found no further instances of it in the manure heaps although it occurs with horse manure and we also observed it in the laboratory experiments. The important conclusion to be drawn from the experiment is that no loss of nitrogen occurs under these conditions. This heap, however, was wholly exceptional. The other compact heaps that we put up all showed a loss of nitrogen, and differed only

in degree from the loose heaps. We shall, therefore, discuss them all together.

Loose heaps. Alongside of the compact heap of cow manure was one that had been thrown together only, and not compacted. The greater degree of looseness allowed a freer access of air, and in consequence the temperature rose higher. The loss of dry matter instead of being 4 % as in the compact heap, was 9 %, and the maximum temperature rose to 16° C. In another experiment heaps of bullock manure were put up which contained less faeces, and therefore could

NITROGEN.

as NH3	19	
as Amide	10	
	15	
	9	
	10	
	11	
	4	
	9	
as other Nitrogen Compounds	71	76	72	62

AT START   KEPT COMPACT AND COVERED   KEPT LOOSE AND COVERED   KEPT LOOSE AND EXPOSED
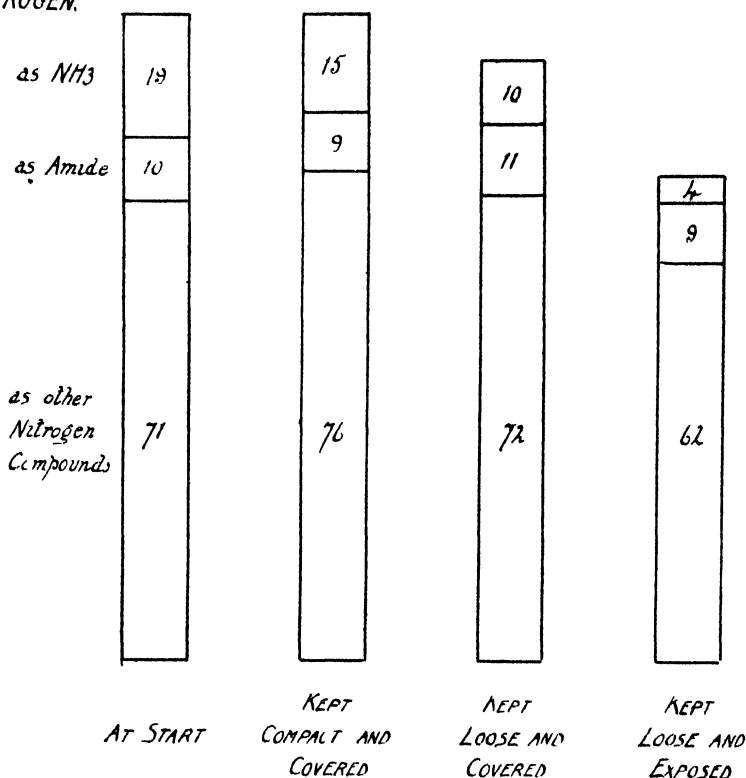
Fig 2   Changes in nitrogen compounds in farmyard manure (cow manure) kept for three months, January 23 to April 30, 1914.

not be compacted nearly so well as the cow manure, so that a greater degree of aeration was attained. The two experiments were not made simultaneously, but there was sufficient similarity in external conditions to allow of a general comparison. The chief results are as follows:

Heaps	Cow manure 3 months Jan. 23— April 30, 1914		The same remade and then left another 3 months April 30— July 21, 1914		Bullock manure 3 months Jan. 7— April 14, 1915		The same 9 months Jan. 7— Oct. 11; 1915	
	Compact	Loose	Compact	Loose	Compact	Loose	Compact	Loose
Highest temperature ...	9°	16°	22·5°	32°	51°	71°	51°	71°
Loss of dry matter %	4	7	9	26	30	35	47·5	45
Loss of nitrogen % ...	Nil	7·5	14	30	26	26	42	32
Of which :—NH_3 ...	4·1	8·4	9	8·5	11	16	15	19
amide ...	0·2	+0·2	Nil	+2	1	Nil	3	Nil
other compounds ...	+4·3	+0·7	5	23·5	14	10	24	13
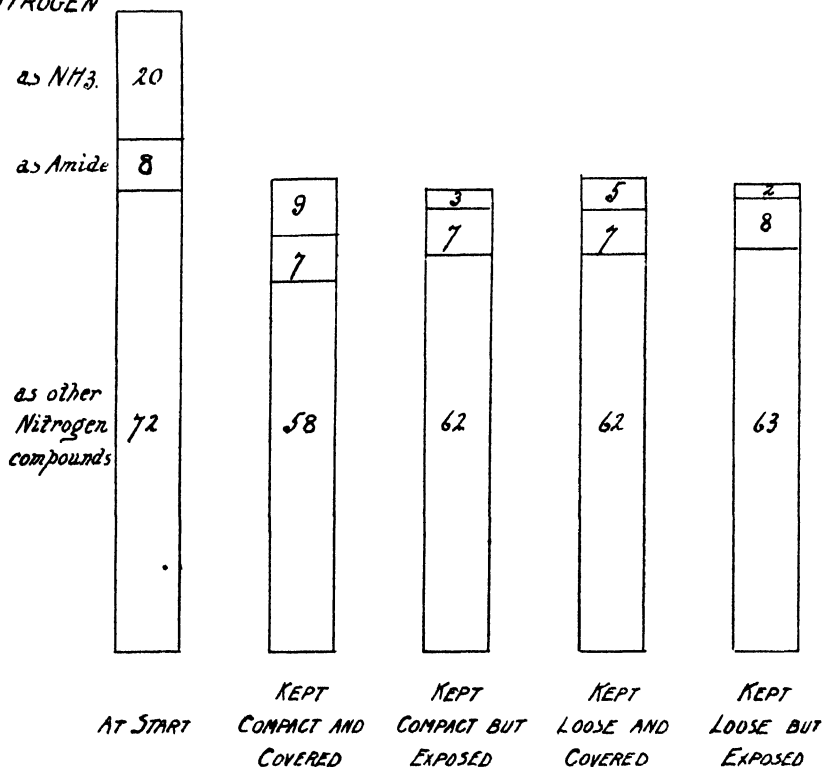
Fig. 3. Changes in nitrogen compounds in farmyard manure (bullock manure) kept for three months, January 7 to April 14, 1915.

The data for the cow manure heaps are set out in Fig. 2 and those for the bullock manure heaps in Fig. 3.

These heaps show a very interesting gradation, starting from the compact cow manure heap in which all the changes are at a minimum,

and ending with the heaps of bullock manure stored for nine months, in which the changes are at a maximum. Up to the end of the three months period the rise in temperature, the loss of dry matter, and the loss of ammonia all vary together, being at a minimum in the compact heap of cow manure, and at a maximum in the loose bullock manure.

The other nitrogen compounds do not follow in the same simple way as the ammonia: the more complex nitrogen compounds show a less regular gradation; there is a gain in the compact cow manure heap, no change in the loose heap, and losses in the bullock manure heaps, but the losses are not proportionate to the loss of dry matter or the temperature. The amides show no gradation at all, and suffer practically no change.

The results for the nine months seem to fall out of line, but in point of fact there has arisen a great difference between the two heaps. The temperature of the loose heap rose during the earlier period to 71° C. at which its microorganic population must have been profoundly altered. The compact heap, on the other hand, only rose to 51° C., and much less change would be produced. In both cases a certain amount of reinfection would undoubtedly take place, but it would probably be limited to the outer layers: a sheltered manure heap through which there is no drainage probably does not allow of easy distribution of infecting organisms. The result is well seen from the temperature curves (Fig. 1 upper portion): after 53 days the loose heap keeps at a *lower* temperature than the compact heap, so that it finally loses less dry matter. Taking the aggregate temperature instead of the maximum the proportionality between temperature and dry matter holds good over the whole period. But the change in nitrogen compounds falls out of line.

If, however, we take only the heaps that did not rise above 51°, in which, therefore, the microorganic population has not been so drastically changed, then the changes in nitrogen compounds fall more closely into line with those of dry matter. The comparison (omitting the amides) now becomes:

Heaps:	Cow manure 3 months		The same re-made and left for another 3 months		Bullock manure, Compact	
	Compact	Loose	Compact	Loose	3 months	9 months
Highest temperature	9°	16°	22·5°	32°	51°	51°
Loss of dry matter ...	4	7	9	26	30	47·5
Loss of nitrogen ...	Nil	7·5	14	30	26	42
Of which:—NH₃ ...	4·1	8·4	9	8·5	11	15
other compounds ...	+4·3	+0·7	5	23·5	14	24

The loss of nitrogen is now seen to be almost proportional to the loss of dry matter, and runs more closely than the ammonia does.

Turning now to the heaps that rose to 71° C.: the further rise in temperature is associated with a correspondingly increased loss of dry matter and of ammonia, but not of total nitrogen; this suffers a smaller loss.

The following rules hold for all the covered heaps we have examined:

The loss of ammonia is determined by the highest temperature attained.

The loss of total nitrogen follows the loss of dry matter so long as the temperature does not rise too high (above 50° C.). But if the temperature goes up much higher (to 70° C.), then the decomposition of the complex nitrogen compounds no longer keeps pace with the other decompositions, and the result is that the loss of nitrogen shows a relative falling off.

From the practical point of view it is important to note that we could only in one case make the heap sufficiently compact to avoid losses of nitrogen.

The loss of nitrogen is not peculiar to our heaps, it is shown in all the compact heaps of which we have been able to find any record; we have set out the data in Table II.

TABLE II. *Loss of nitrogen during storage of farmyard manure as recorded by other experimenters.*

Woburn experiments[1].			1899	1900	1901
Loss of nitrogen	...	...	30·7	17·3	18·9

Berry's experiments[2].					
Loss of nitrogen	...	...	19 9	—	—
Loss of dry matter	...	...	11·0	—	—

Under cover, cement floor.

Wood's experiments[3].		Animals receiving roots and hay only	Roots, hay and cake
Loss of nitrogen	... ...	10·6	26·9
Loss of dry matter	... ...	16·2	18·6

Manure left undisturbed in feeding box.

Goodwin and Russell's experiments[4].		Bullock receiving	
		Rich cake	Poor cake
Loss of nitrogen	... ...	15·4	13·3

[1] See *Journ. Roy. Agric. Soc.* 1902, **63**, 85–87.
[2] Berry, *West of Scot. Bull.* No. XIV. 1914.   [3] Wood, This *Journ.* 1907–8, **2**, 207.
[4] *Journal S.E. Agric. Coll.* 1905, No. 14, 187.

Effect of drying out of the heap.   The effect of drying out of the heap is well seen in the experiment at Woking, where some mixed manure was left in a closed shed protected from wind and weather from November 1st, 1913, to May 20th, 1914, during which time its moisture content fell from 76·8 % to 72·6 %. The difference in condition of the manure was more than these figures indicate; with 76·8 % of water the heap appeared considerably wetter than with 72·6 %. The most characteristic reaction is the formation of nitrates, which, in our experience, only occurs when the moisture content falls below a certain critical amount (see p. 541). In the Woking experiment the nitric nitrogen formed as much as 9 % of the original total nitrogen; this, however, is quite exceptional: more usually only a small quantity is detected.

. The loss of ammonia amounts to 12 % of the original total nitrogen, and is therefore greater than the nitrate formed: there is also a loss of more complex nitrogen compounds, but not of amide.

Another instance is furnished by the heap of cow manure also stored under cover, but exposed to wind for the long period, Nov. 29th, 1915, to Jan. 26th, 1917, in which the changes on the whole are similar. though the formation of nitrate is less.

The results are as follows:

	Mixed manure at Woking (sheltered from wind)		Cow manure, Rothamsted (not sheltered from wind)	
	at start Nov. 1, 1913	at end May 20, 1914	at start Nov. 29, 1915	at end Jan. 22, 1917
Percentage of moisture	76 8	72·6	82 0	71·0
Dry matter	100	74	100	79
Nitrogen	100	92	100	71
Of which :—NH_3 ...	16	4	19	3
nitrate	0	9	0	Present
amide ...	8	8	7	5
other compounds	76	71	74	63

The evaporation of water is naturally accompanied by an evaporation of ammonia, which, therefore, becomes a source of loss of nitrogen. But the amount of water evaporated is not great in proportion to its total quantity, and unless there was relatively more evaporation of ammonia the actual amount lost would only be small. In order to find out whether the continued maintenance of the moisture content of the heap would help to retain the ammonia and the total nitrogen the following experiment was carried out.

Effect of watering the heap.   One of the heaps of cow manure kept loose under cover was periodically watered from a can, sufficient water

being added to keep the heap perpetually moist without, however, putting on any excess to drain away. The result may have been to diminish the evaporation of ammonia, but it certainly did not diminish the loss of nitrogen: on the contrary the loss rose from 7·5 to 13·6 %, all of which fell on the more complex compounds:

	Watered	Left unwatered
Highest temperature	15°·5	16°·5
Loss of dry matter, original dry matter being - 100	5·1	7·4
Loss of nitrogen, original nitrogen being −100 ...	13 6	7·5
Of which : - NH₃ lost	7·3	8·4
amide gained	0·6	0·2
other compounds lost	6·9	+0·7

Effect of exposure. The heaps already described were all kept under cover. A similar set was put up close by, but exposed on all sides to wind, rain, and sun. The first effect was to cause the outer layer to
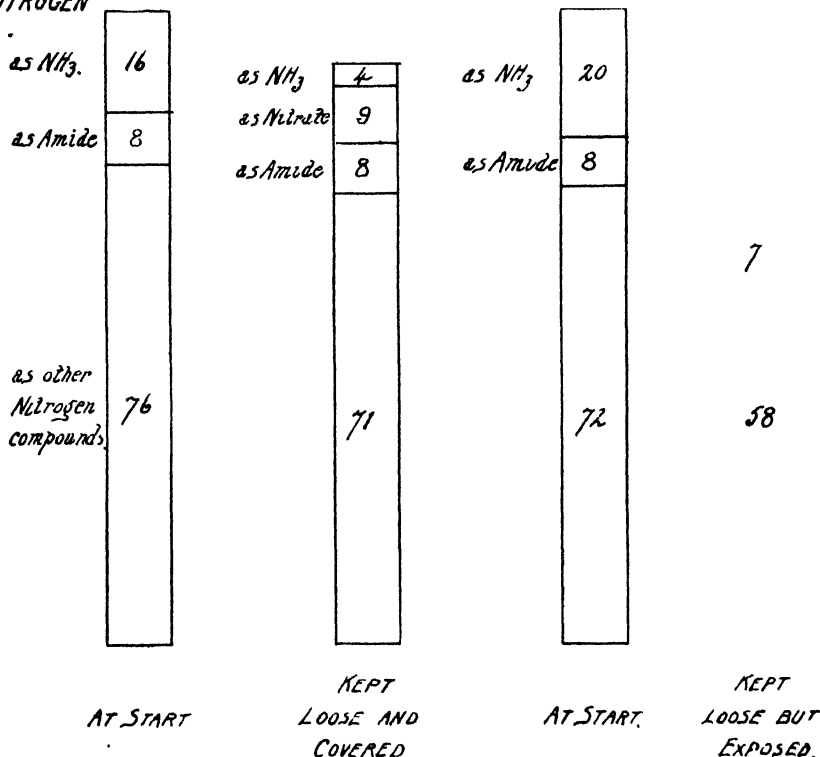
Fig. 4. Changes in nitrogen compounds in farmyard manure (mixed) kept for six months, at Woking.

mat together into a kind of thatch which, had it been perfect, would no doubt have shielded the rest of the heap. But in its imperfect state it allowed rain to get in, much of which flowed away again as a stream of black liquid. This, of course, complicates the matter, and it is not surprising that the exposed heaps do not show the relationship between temperature and losses that are exhibited in the sheltered heaps. The temperature of these exposed heaps did not rise so high as that of the covered heaps excepting in one case, but the loss of dry matter was considerably higher. The total loss of nitrogen compounds, however, did not follow in the same way, and was sometimes no more than in the sheltered heaps in spite of the difference in loss of dry matter: this was particularly the case with the bullock manure during the first three months of storage. The loss of ammonia was usually much greater, excepting only in two cases, while the loss of other nitrogen compounds was sometimes actually less. This is shown in Table III, other results obtained with mixed manure are given in Fig. 4.

TABLE III. *Effect of shelter on the amount of loss from decomposing farmyard manure.*

Cow manure stored loosely for

	3 months		Re-made and left for 3 months	
	Exposed	Covered	Exposed	Covered
Highest temperature	21°	16°	29°	32°
Loss of dry matter	21	7	28	26
Loss of nitrogen	25	7·5	13	30
Of which:—NH$_3$	15	8·4	3	8·5
other compounds ...	9	+0·7	12	23·5

Bullock manure.

	Compact 3 mths		Compact 9 mths		Loose 3 mths		Loose 9 mths	
	Exposed	Covered	Exposed	Covered	Exposed	Covered	Exposed	Covered
Highest temperature ...	40°	51°	40°	51°	55°	71°	55°	71°
Loss of dry matter ...	39	30	60	47·5	41	35	60	45
Loss of nitrogen	28	26	50	42	27	26	51	32
Of which:—NH$_3$	16	11	17	15	16	16	17	19
„ amide	Nil	1	3	3	Nil	Nil	4	Nil
„ other compounds	12	14	30	24	11	10	30	13

In these tables the original dry matter is put -100 as also is the original nitrogen.

There is evidence here of two opposing factors: the rain washes out soluble matter, but it also keeps down the temperature and thereby reduces decomposition of the nitrogen compounds and loss of ammonia

relative to the total change. The final losses of nitrogen, therefore, are not so great as appears from the shrinkage of the heap, although on the absolute they are generally greater than the losses from the covered heaps.

The non-nitrogenous constituents of the dry matter thus appear to suffer relatively more than the nitrogen compounds as the result of exposure. The result seems to be fairly general: it was obtained by Maercker at Halle in 1897, and in Scotland by Berry.

	Maercker* Halle, 1896		Berry, Glasgow. 1914	
	Sheltered	Exposed	Sheltered	Exposed
Loss of dry matter, per cent. ...	1	21	11	23
Loss of total nitrogen per cent.	37	37	20	28
Loss of NH$_3$ per cent.	74	81	75	78

* This experiment is really a comparison between manure stored under the animals in the "Tiefstall," and manure thrown out daily on to two heaps, one exposed and one sheltered. The figures for the loss of nitrogen are absolute determinations, the loss from the "Tiefstall" manure being only 13 per cent. The values for dry matter loss, on the other hand, are relative only and simply represent the excess over and above the losses in the "Tiefstall." The result shows that the losses of nitrogen have been greater than those of dry matter. See *Landw. Jahrbüch.* 1898, **27**, 215

Direct field experiments have shown that the exposed heaps have less crop producing power than the sheltered heaps. Our own are recorded on p. 505: 10 tons of the sheltered manure (the compact bullock manure, stored three months) gave an increase over the control of 76 % in the yield of potatoes, while an equal dressing of the exposed manure gave only 44 % increase: for the corresponding loose heaps the increases were 73 % from the sheltered, and 57 % from the exposed manure. This was on equal weights of the final manure: had we taken equal weights of the original manure we should have expected even more striking results because of the difference in dry matter. The 1916 experiments with potatoes, and both experiments with wheat, give similar results. In like manner Berry found that 30 tons of the sheltered manure gave an increase of 57 % in yield of potatoes, and 45 % of turnips, while an equal dressing of the exposed manure gave only 49 % increased yield of potatoes, and 38 % of turnips, again using equal weights of final, and not of original manure.

Effect of summer storage. The nine months experiments lasted over the summer, and the columns in Figs. 5 and 6 show how serious the losses were then. The question is one of great practical importance on farms where there is much grass land in proportion to arable, and

whete, therefore, the manure collected during winter is more than is wanted for the roots.

How far can the losses go? Reference to the tables, and still more easily to the figs. show that in general the loss of nitrogen compounds is rapid at first, and falls off afterwards.   Thus, in the cow manure (Fig. 6) the nitrogen as ammonia rapidly falls from 19 to 4, then to 1, while the more
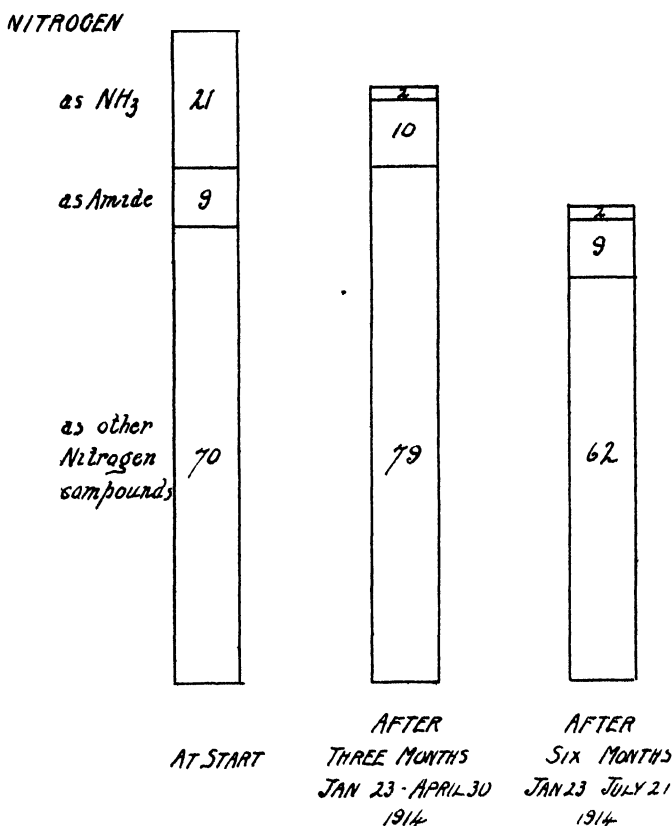
Fig. 5.   Changes in nitrogen compounds in farmyard manure (horse manure) in the open in loose heaps.

complex compounds fall from 73 to 65, then to 57, and finally after nine months to 55: the amides on the other hand remained practically constant.   The total nitrogen at the end was only 62 % of the quantity originally present, making a loss of 38 %.   In the bullock dung left for the same period the losses are finally greater, the total nitrogen falling 50 %.

Looking over the results it will be observed that in no case does the nitrogen present as complex compounds become reduced to less than 50 % of the original nitrogen, and even before it falls so low the rate of loss becomes very small: further, the total loss of nitrogen is rarely more than 40 %,—the remaining 60 % apparently being more resistant than the rest. Cow manure, bullock manure, and the mixed manure at Woking, all show these relationships.
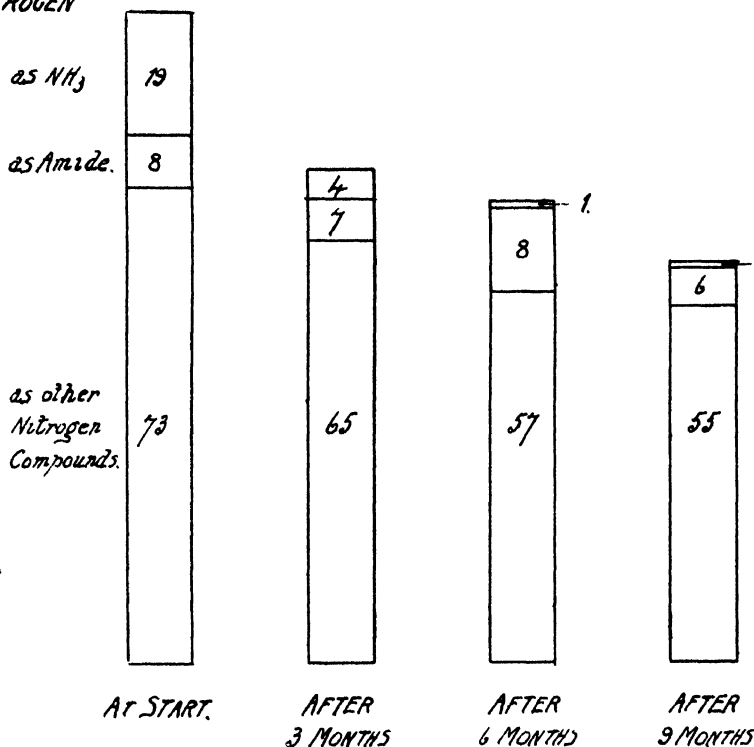
Fig. 6. Changes in nitrogen compounds in farmyard manure (cow manure) stored in loose heaps in the open for varying periods.

ON THE REACTIONS TAKING PLACE IN THE HEAP.

1. *The study of similar cases.*

The changes observed in the nitrogen compounds are:
1. Loss of ammonia,
2. Almost always a loss of total nitrogen,
3. Almost always a loss of complex nitrogen compounds.

The two obvious ways in which nitrogen may be lost from the heap are:

1. By the washing out of soluble nitrogen compounds.
2. By the volatilisation of ammonia or other compounds. .

Washing out is insufficient to account for all the loss because it does not operate under cover, and yet loss of nitrogen always occurred here, and was sometimes as great as in the open.

It is much more difficult to decide whether volatilisation of ammonia accounts for everything else. One or two facts, however, would be difficult to explain on this view.

Ammonia does not easily volatilise from farmyard manure, even the exposed heaps retaining a good deal of it, not infrequently indeed as much as in the sheltered heaps. Nor is there much volatilisation of water except from the outer crust: usually the moisture only falls by a few per cents. of its total quantity: unless ammonia volatilises more readily and to a greater extent the loss would be insufficient to account for the facts.

Again, the watered heap (p. 516) showed no more loss of ammonia than the unwatered heap, nor did it accumulate ammonia, yet it lost considerably more nitrogen.

Thus we are driven to seek for some other cause for the loss of nitrogen.

It is still more difficult to follow the changes in the complex nitrogen compounds of the heap. Little can be gathered from the analytical data beyond the fact that they disappear. We know, however, that the process is brought about by microorganisms; our first step is therefore to summarise the results obtained in the laboratory investigations on the bacterial decomposition of protein, and then to consider another important case of decomposition,—the reactions occurring during sewage purification,—to see what light they throw on the change in the manure heaps.

The bacterial decomposition of protein under laboratory conditions.

Considerable work has been done in late years in studying the bacterial decomposition of protein. An extensive literature on this subject has grown up most of which, fortunately, has been summarised in Barger's monograph[1]; it is, therefore, unnecessary for us to do more

[1] "The Simpler Natural Bases." G. Barger, *Longman's Monographs on Biochemistry,* 1914.

than indicate the leading lines. The first stages in the bacterial decomposition break the protein down to amino-acids; the change seems to be identical with the ordinary laboratory decomposition brought about by sulphuric acid and other hydrolysing agents.

The special feature of bacterial decomposition is that these amino-acids break down still further, and the change may go on in two directions:

1. Deaminisation, i.e. the elimination of ammonia. This is a very common change and is produced under both aerobic and anaerobic conditions; indeed it is not solely a bacterial process, but seems to be a widespread property of the living cell[1]. We are here concerned only with deaminisation by bacteria, a subject which has been much investigated[2]. Of the aerobic processes known to bring it about two have been described by Dakin[3]:

$$R . CH . NH_2 . COOH + O_2 = R . COOH + CO_2 + NH_3$$
$$R . CH . NH_2 . COOH + H_2O = R . CH . OH . COOH + NH_3$$
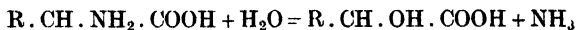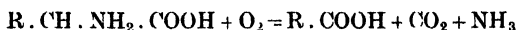
whilst another which, so far as is known, is confined to yeast, was discovered by Ehrlich[4]:

$$R . CH . NH_2 . COOH + H_2O = R . CH_2OH + CO_2 + NH_3.$$
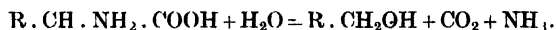
Besides these there are the following anaerobic processes, one accompanied by reduction:

$$\begin{array}{ccc} R & & R \\ | & & | \\ CHNH_2 + 2H & - & CH_2 + NH_3 \\ | & & | \\ COOH & & COOH \end{array}$$
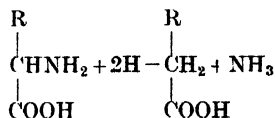
and another recently studied by Raistrick[5] in which there is no reduction:

$$R . CH_2 . CHNH_2 . COOH \rightarrow R . CH : CH . COOH + NH_3.$$

[1] See "The Physiology of Protein Metabolism," Cathcart, *Longman's Biochemical Monographs*, 1912, pp. 49–55

[2] E.g. Brasch and Neuberg, *Biochem. Zeit.* 1908, **13**, 299; and 1909, **22**, 403. Neuberg, *ibid.* 1909, **18**, 424 et seq. and 1909, **20**, 450 et seq. Borchardt, *Zeit. f. physiol. Chem.* 1909, **59**, 96.

[3] Dakin, *J. Biol. Chem.* 1908, **4**, 63.

[4] Ehrlich, *Zeitsch. Verein Rübenzucker Ind.* 1905, 539–67.

[5] *Biochem. J.* 1917, **11**, 71.

2. Decarboxylation, i.e., the elimination of CO_2;

$$\begin{array}{cc} R & R \\ | & | \\ \overset{|}{C}HNH_2 = \overset{|}{C}H_2NH_2 + CO_2 \\ | \\ COO\ H \end{array}$$
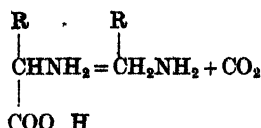
or, instead of CO_2, formic acid may be split off:

$$\begin{array}{cc} R & R \\ | & | \\ \overset{|}{C}H.NH_2 + H = \overset{|}{C}H_2NH_2 + H.COOH \\ | \\ COO\ H\ \ H \end{array}$$
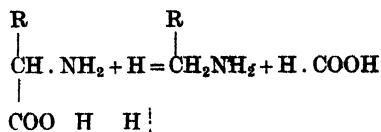
Unlike deaminisation, decarboxylation is an anaerobic process, any exception being explained by assuming the presence of some obligate anaerobe[1]. It is the characteristic feature of putrefaction: the nitrogenous bases or *amines* produced being typical putrefaction products.

We have, therefore, this fundamental distinction:

Under *aerobic* conditions the general type of action is that ammonia splits off and a complex acid is left.

Under *anaerobic* conditions two types of action may occur: ammonia may be split off leaving a complex acid as before, or carbon dioxide may be split off leaving a complex amine.

When the aeration conditions change the products change also: a molecular grouping which is stable under anaerobic conditions may prove unstable as soon as air is admitted: e.g. p-hydroxy-phenyl-propionic acid, which breaks down further[2] to p-cresol and phenol, etc. Similarly, groupings stable under aerobic conditions, e.g. hydroxy-acids, do not seem to form under anaerobic conditions.

It is not known precisely what conditions determine whether deaminisation or decarboxylation shall take place, but usually both go on simultaneously, and deaminisation preponderates. But if we regard the decomposition of protein as a preliminary process for the nutrition of plants it would appear that deaminisation is the type of decomposition at which the agriculturist should aim. The acids which are simultaneously formed give rise to calcium salts which probably break down to form calcium carbonate in the soil, while the ammonia is

[1] Bienstock, *Archiv f. Hygiene*, 1899, **36**, 355–89; 1901, **39**, 390–427. Rettger, L. F. *J. Biol. Chem.* 1906, **2**, 71–86; 1907, **4**, 45–55; and 1912, **13**, 341–46.

[2] See Barger, *loc. cit.* p. 8.

rapidly oxidised to nitrates. On the other hand decarboxylation gives only amines, some at least of which are resistant to further bacterial action, e.g. putrescine and cadaverine, which are among the commonest putrefaction products. It is not known how these would decompose in the soil: we may safely assume that they would ultimately undergo oxidation, but the best results may be anticipated from the deaminisation process. Unfortunately, as already stated, the conditions which determine the two processes are not known.

The important point for our present investigation is that in the bacterial decompositions of complex nitrogen compounds so far studied the first products· are amino-acids, which subsequently give rise to ammonia, and, in anaerobic conditions, to amines as well.

The decompositions occurring during the purification of sewage.

These decompositions afford another example of the biochemical decomposition of complex nitrogen compounds, and the investigations bring out three facts of considerable importance for our problem:

1. The decomposition of complex nitrogen compounds always gives rise to ammonia.

2. Under anaerobic conditions the conversion is quantitative, the ammonia being exactly equivalent to the amount of complex nitrogen compounds decomposed.

3. Under aerobic conditions, on the other hand, the ammonia and nitrate formed are not equivalent to the complex compounds decomposed, but there is always a loss.

The decomposition under anaerobic conditions is well shown in two very careful experiments, each lasting over two years, made by the Royal Commission on Sewage Disposal on the working of septic tanks at Exeter and Ilford[1]. The conditions obtaining in the tanks are strictly anaerobic. During the two years the amounts of dry matter entering the tanks, and the amounts decomposed were:

	Exeter	Ilford
Suspended solids in sewage (lbs.) ...	126,801	38,824
Solids in tank sludge...	47,842 ⎱ 96,379	11,756 ⎱ 27,226
Solids escaping in tank liquor ...	48,537 ⎰	15,470 ⎰
Solids digested	30,422	11,598
Percentage of solids digested ...	24·0	29·9

[1] McGowan, Houston, Frye and Kershaw, Royal Commission on Sewage Disposal, 5th Report, Appendix IV. 1910, pp. 249–51

There was therefore considerable breaking down of organic matter. Much of the nitrogenous matter was decomposed with formation of ammonia, the gain in ammoniacal nitrogen being:

	Exeter	Ilford
Ammoniacal nitrogen in sewage (lbs.)	14,711	8,311
„ „ „ septic tank liquor ...	16,452	9,020
Increase in ammoniacal nitrogen	1,741	709
Percentage increase	11·8	8·5

But there was no loss of nitrogen; the total amount remained practically unchanged:

Total nitrogen in lbs.		Exeter		Ilford
In sewage entering septic tank		22,915		11,052
In liquor leaving septic tank	21,372 }	22,499	10,735 }	11,176
In sludge deposited in septic tank ...	1,127)		441)	
Loss or gain of nitrogen		−416		+124
Per cent.		−1·82		+1·12

Under aerobic conditions, however, a loss of nitrogen sets in. This is shown in an experiment made by the Sewage Commission at Accrington[1] in which sewage was run by means of a rotating sprinkler from the septic tank on to a percolating filter made of clinker. The persistence of the aerobic conditions is demonstrated by the fact that the effluent remained saturated with dissolved oxygen. The experiment continued altogether for three years, and was divided into five periods; the results of all show considerable agreement so that it is not necessary for us to transcribe more than two. The figures are stated as parts per 100,000, but as the volume of sewage remained approximately constant they are proportional to the total weights.

Loss of nitrogen in aerobic decomposition of sewage.

	First period 15 months		Second period 7 months	
	Entering filter	Leaving filter	Entering filter	Leaving filter
Nitrogen as complex compounds ...	1·37	0·81	2·20	0·50
„ „ ammonia	4·75	0·98	3·86	0·24
„ „ nitrate	0·00	2·46	0·00	3·25
Nitrogen lost	—	1·87	—	2·07
Total	6·12	6·12	6·06	6·06
Percentage loss of nitrogen	—	30·6	—	34·2

It will be observed that the loss of nitrogen now exceeds 30 %, whereas in the preceeding experiments it was *nil*.

[1] McGowan and Frye, *ibid.* pp. 52–72.

No account is taken here of the solid matter deposited on the filter, but over these long periods it probably has no great disturbing effect on the figures.

Variations in oxygen supply caused variations in the loss of nitrogen but usually in an opposite direction: thus when the oxygen supply was increased by diluting the sewage with an equal volume of tap-water the loss was reduced to about 20 %, while breaks in the continuity of aeration, such as are obtained when the liquid was run on to the contact beds, caused the loss to increase to 50 %[1].

These experiments do not tell us what became of the nitrogen. But in an earlier experiment by Letts[2] it was shown that the amount of gaseous nitrogen dissolved in a contact bed effluent was greater than in the original sewage, the increase amounting to 16–20 % of the missing nitrogen. There was therefore an actual production of gaseous nitrogen during the process. The figures do not balance, but this could hardly be expected: it is unlikely that the whole of the gaseous nitrogen would remain dissolved in the effluent, and in any case part of the loss of nitrogen from the sewage must be attributed to the hosts of insects, worms, etc., which breed in it, and then move off elsewhere.

[1] Thus the losses on the addition of tap-water were:

Diluted sewage (equal volume of tap-water added)

	1st period 15 months	2nd period 7 months	3rd period 2 months	4th period 4 months	5th period 8 months
Rate of filtration per gall. per cubic yard per day ...	200	100	250	400	500
Nitrogen in original liquid ...	3·32	3·07	2·64	2·77	2·75
Nitrogen in effluent	3·09	2·44	2·00	2·23	2·02
Nitrogen lost	0·23	0·63	0·64	0·54	0·73
Nitrogen lost, per cent. ...	6·9	20·5	24·2	19·5	26·5

Those on the contact bed were:

	1st period, 15 months		2nd period, 7 months	
	Entering bed	Leaving bed	Entering bed	Leaving bed
Nitrogen as complex compounds	1·24	0·68	1·78	0·64
,, ,, ammonia ...	4·86	1·56	4·40	1·21
,, ,, nitrate	0·00	0·97	0·00	0·98
Loss	—	2·89	—	3·34
Total	6·10	6·10	6·18	6·18
Nitrogen lost, per cent.... ...	—	47·4	—	54·0

The figures are all shown as per 100,000 of sewage but as the volume of sewage did not change they are proportional to the actual weights involved.

Some of the missing nitrogen in this case is to be found in the sludge which deposits each time the contact bed is filled.

[2] Report to the Corporation of Belfast on the purification of the Belfast Sewage (Baird, Belfast, 1908). Also, 5th Report of the Sewage Commission. Appendix VI. pp. 171–94.

THE REACTIONS TAKING PLACE IN THE HEAP.

II. *The decomposition of farmyard manure under carefully controlled conditions in the laboratory.*

We have just seen that the bacterial decomposition of protein in the laboratory involves the formation first of amino-acids and then of ammonia. This reaction proceeds under both aerobic and anaerobic conditions, but in the latter case amines are formed as well. The complex nitrogen compounds of sewage (which are presumably of the same nature as protein, or contain the protein groupings) also decompose under anaerobic conditions to yield ammonia and presumably amines: we may suppose therefore that the reaction is similar to the above, and that amino-acids are formed as intermediate products.

Under aerobic conditions the same reaction apparently goes on, but it proceeds further and gives rise to a certain amount of nitrate. But a new reaction also sets in whereby some nitrogen is liberated.

Thus, the sewage decompositions seem to be entirely on a par with the bacterial decomposition of protein. But the decompositions in the manure heap do not: there is, for example, no accumulation of ammonia or anything to indicate that the complex nitrogen compounds break down through the ammonia stage at all. It is true that the end products are the same as in the aerobic stage of sewage decomposition, indicating that the reactions may be the same also, but the parallel cannot be pressed too far until it is known whether decomposition under strictly anaerobic conditions would also resemble that of sewage. However well the manure heap is compacted we have never found proof that this was the case.

As it is difficult to control the conditions in a manure heap we carried out the next set of experiments in the laboratory. The manure was put up in bottles or jars and sealed down tightly for the anaerobic experiments, or exposed to a curent of washed and purified air for the aerobic experiments, acid traps being provided to retain any ammonia carried over. The aerobic and anaerobic experiments were made side by side to allow of a strict comparison of the results, but it will be convenient to describe them separately.

Anaerobic storage. The results obtained under anaerobic conditions are set out in Table IV and Fig. 7.

In all cases there is a breaking down of some of the complex nitrogen compounds.

In all cases, except one, there is an increase in the amount of ammonia: the exception is a sample of manure that had been stored some time before the experiment began, and had lost most of its ammonia.

As the temperature rose this action became more pronounced, and at 26° C. the loss of complex nitrogen compounds and the gain of ammonia was considerably greater than at about 15°.

NITROGEN.

as NH₃   28
as AMIDE   8

as other
Nitrogen   64
compounds.

          29
           9

          57

          44

           7

          47

AT START   STORED   STORED
            AT 6°C FOR   AT 26°C FOR
            50 DAYS.   50 DAYS
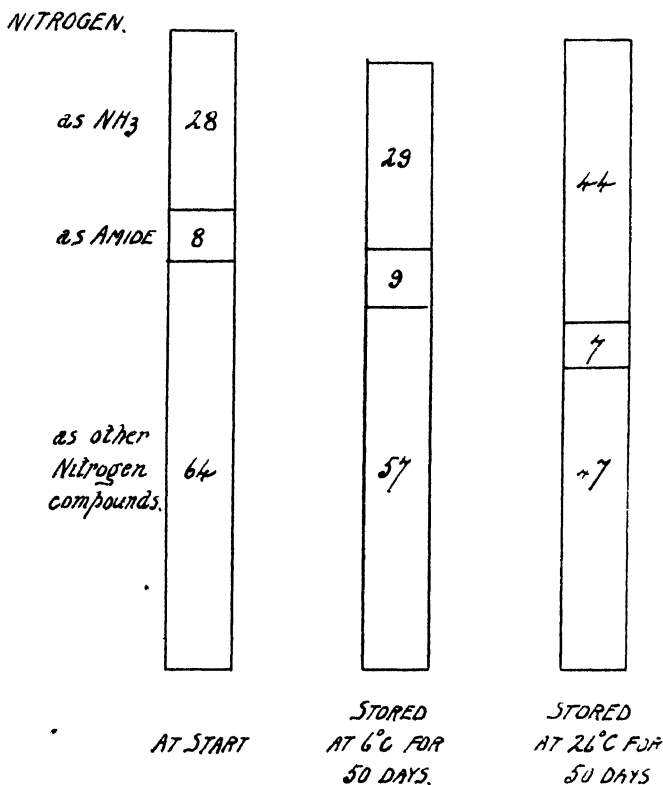
Fig. 7. Changes in nitrogen compounds in farmyard manure (bullock manure) stored in the laboratory under anaerobic conditions at different temperatures.

In two out of four cases the gain in ammonia nearly balances the loss of complex nitrogen compounds: this happened when the manure was most closely packed. With looser packing, where some air would be left in the early stages, the ammonia produced was not equivalent to the complex nitrogen compounds broken down, and there was a deficit on the total nitrogen amounting to 4·5 %. With tighter packing and strictly anaerobic conditions there was no appreciable loss.

It will be seen that these changes are precisely similar to those observed in the laboratory investigations on protein, and in the anaerobic decomposition of sewage. The apparent discrepancy between these and the manure heap is therefore not due to any peculiarity of the manure, but arises solely from the conditions of storage in the heap.
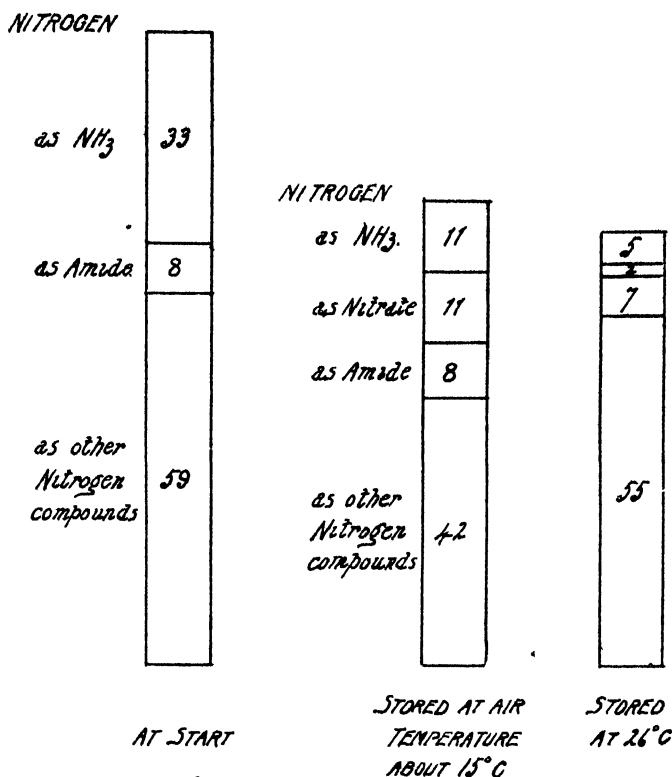
Fig. 8.   Changes in nitrogen compounds in farmyard manure (bullock manure) stored in the laboratory under aerobic conditions at different temperatures.

Aerobic storage.   The results obtained under aerobic storage are given in Table V, and set out in Fig. 8.   There is no appreciable gain in ammonia, in one case there is an actual loss.   But a new feature appears in these experiments: the nitrogen at the end is no longer equal to that at the beginning, but is notably less: there has been, therefore, a loss of nitrogen.   This shows that some change goes on here which did not occur under anaerobic conditions.   The aggregate amount of complex nitrogen compounds broken down increases when air is admitted: it is possible that the whole is converted into ammonia, and if so there

TABLE IV. *Changes in small quantities of manure stored in the laboratory. Anaerobic conditions.*

Expt. 1. Bullock manure, Rothamsted, April, 1914.

Expt. 2. Mixed manures: bullock, horse and pig, Woking. Made in May, stored till June, then put up for experiment.

		Experiment 1		Experiment 2	
	Initial	After storage anaerobically for 203 days	Initial	After storage anaerobically for 112 days	
Total weight, grams	5641	5537	5575	5585	
Composition per cent.					
Dry matter	28·2	23·8	22·7	22·3	
N as ammonia	·081	·170	·023	·011	
N as amide	·063	·016	·037	·047	
N as other compounds ...	·472	·435	·554	·545	
Total nitrogen	·616	·621	·614	·603	
		Loss or gain		Loss or gain	
Amount of dry matter, parts by weight	100	83	−17	100	95
Of 100 parts of N originally present, there are :—					
As ammonia	13·1	27·1	+14	3·7	1 7
As amide	10·2	2·6	−7·6	6·0	7·4
As other compounds ...	76·7	69·2	−7·5	90·3	86·2
Total nitrogen	100	98·9	−1·1	100	95·3

(right-most "Loss or gain" column values: Experiment 2 — weight −5; As ammonia −2; As amide +1·4; As other compounds −4·1; Total nitrogen −4·7)

In Experiment 2 the manure was less closely packed than in Experiment 1, so that a certain amount of air was present in the early stages of the decomposition.

Expt. 3. Manure stored at air temperature and at 26° C.

	Initial	Stored for 50 days at air temperature		Stored for 50 days at 26° C	
Total weight, grams	{491 {501	487		493	
Composition per cent.					
Dry matter	25·2	24·9		23·9	
Nitrogen as NH₃	·203	·215		·321	
„ „ amide	·057	·064		·055	
„ „ other compounds	·472	·425		·349	
Total nitrogen	·732	·704		·725	
		Loss or gain		Loss or gain	
Amount of dry matter, parts by weight	100	98	−2	93	−7
Of 100 parts of N originally present there are:					
As NH₃	27·8	29·2	+1·4	43·1	+15·3
„ amide	7·8	8·6	Nil	7·4	Nil
„ other compounds	64·4	57·5	−6·9	46·8	−17·6
Total nitrogen	100	95·3	−4·7	97·3	−2·7

TABLE V. *Change in small quantities of manure stored in the laboratory.*
Aerobic conditions.

Expt. 1.   Bullock manure, Rothamsted, April, 1914.

	Initial	Aerobically for 194 days, after storage anaerobically for 210 days	
Total weight, grams	5868	5614	
Composition per cent.			
Dry matter	28·2	22·3	
Nitrogen as ammonia	·081	·022	
„ „ nitrate	—	* trace	
„ „ amide	·063	·043	
„ „ other compounds	·472	·392	
Total nitrogen	·616	·457	
			Loss or gain
Amount of dry matter, parts by weight ...	100	90	− 10
Of 100 parts of N originally present there are:			
As ammonia	13·1	3·4	− 9·7
„ nitrate	—	—	—
„ amide	10·2	6·7	− 3·5
„ other compounds	76·7	60·3	− 16·4
Total nitrogen	‚100	70·4	− 29·6

Expt. 2.   Mixed manure: bullock, horse and pig, Woking.   Made in May, stored till July, then put up for experiment.

	Initial	Aerobically for 112 days		Aerobically for 56 days, after storage anaerobically for 56 days	
Total weight, grams	5926	5670		—	
	6153	—		5897	
Composition per cent.					
Dry matter	22·7	20·7		21·6	
Nitrogen as ammonia	·023*	·036		·017	
„ „ nitrate	—	—		—	
„ „ amide	·037	·019		·046	
„ „ other compounds	·554	·481		·486	
Total nitrogen	·614	·537		·551	
			Loss or gain		Loss or gain
Amount of dry matter, parts by weight	100	96	− 4	96	− 4
Of 100 parts of N originally present there are:					
As ammonia	3·7	5·6	+ 1·9	2·6	− 1·1
„ nitrate	—	·2	+ 0·2	·3	+ 0·3
„ amide	6·0	3·0	− 3·0	7·2	+ 1·2
„ other compounds	90·3	74·9	− 15·4	75·9	− 14·4
Total nitrogen	100	83·7	− 16·3	86·0	− 14·0

* When the sample was first drawn in May it contained 0·15 % of ammonia, but during storage this fell to the amount here given.

For full analyses and descriptions see Appendix.

Expt. 3. Effect of temperature on the aerobic decomposition of large and small quantities of bullock dung.

	Large quantity (500 grams)							
	Sample A				Sample B			
Kept at 15° C.	At start	At end	Differ-ence	Loss or gain %	At start	At end	Differ-ence	Loss or gain %
ght of wet manure, grms.	500·5	496·3	−4·2	− 0·8	485·5	561·3*	+75·8	—
matter	119·4	116·1	−3·3	− 2·8	115·8	112·8	− 3·0	− 2·6
moniacal nitrogen ...	1·26	0·50	−0·76	− 19·7	1·22	0·32	− 0·90	− 24·1
ide nitrogen ...	0·31	0·08	−0·23	− 6·0	0·30	0·35	+ 0·05	+ 1·3
ous or nitric nitrogen ...	none	0·38	+0·38	+ 9·9	none	0·12	+ 0·12	+ 3·2
er nitrogen	2·28	1·62	−0·66	− 17·2	2·21	2·08	− 0·13	− 3·5
al nitrogen	3·85	2·58	−1·27	− 33·0	3·73	2·87	− 0·86	− 23·1
moniacal nitrogen recovered								
i acid trap	—	0·07	—	1·8	—	0·07	— .	1·9
Nett loss of nitrogen ...	—	—	—	31·2	—	—	—	21·2
Kept at 26° C.								
ght of wet manure ...	460·1	456·1	−4·0	− 0·9	427·0	422·6	− 4·4	− 1·0
matter	109·8	106·3	−3·5	− 3·2	101·9	94·2	− 7·7	− 7·6
moniacal nitrogen... ...	1·15	0·24	−0·91	− 25·7	1·07	0·08	0·99	− 30·2
ide nitrogen	0·29	0·27	−0·02	− 0·6	0·26	0·22	− 0·04	− 1·2
ous or nitric nitrogen ...	none	0·06	+0·06	+ 1·7	none	0·06	+ 0·06	+ 1·8
er nitrogen	2·10	1·94	−0·16	− 4·5	1·95	1·85	− 0·10	− 3·0
al nitrogen	3·54	2·51	−1·03	− 29·1	3·28	2·21	− 1·07	− 32·6
moniacal nitrogen recovered								
i acid trap	—	0·20	—	5·6	—	0·20	—	6·1
Nett loss of nitrogen ...	—	—	—	23·5	—	—	—	26·5

* About 80 c.c. of water accidentally sucked back in this bottle.

The percentage losses or gains of the ammoniacal, amide and other forms of nitrogen are expressed as percentages of the total nitrogen.

Small quantity (5 grams)

	Kept at 15° C.	Sample A	Sample B
Weight of wet manure, grms	... ...	5·012	5·048
Dry matter	... ...	1·196	1·200
Total nitrogen at start, mgs.	... ...	38·5	38·8
,, ,, at end	... ...	23·6	27·3
,, ,, lost	... ...	14·9	11·5
Ammoniacal nitrogen recovered in acid trap		5·0	3·4
Nett loss of nitrogen		9·1 = 25·7 %	8·1 = 20·9 %

	Kept at 26° C.	Sample A	Sample B
Weight of wet manure, grms.	... ...	5·008	5·069
Dry matter	... ...	1·194	1·204
Total nitrogen at start, mgs.	... ...	38·5	39·0
,, ,, at end	... ...	27·5	28·6
,, ,, lost	... ...	11·0	10·4
Ammoniacal nitrogen recovered in acid trap		3·3	3·8
Nett loss of nitrogen		7·7 = 20·0 %	6·6 = 16·9 %

would be more than under anaerobic conditions.   But any such increased ammonia production is wholly masked by the new change whereby nitrogen is lost.

An increase in temperature to 26° causes some increase in the amount of decomposition of the dry matter but not in the loss of nitrogen. There is, however, an increased loss of ammonia and no indication of a gain as in the anaerobic experiments.   Similar results were obtained whether we worked with 5 grams of manure or 500.

The fact that nitrogen is lost under aerobic conditions was also shown by Sjollema and de Wildt in their experiments at Gronigen during the years 1906–10[1].   They made up an artificial mixture of faeces and urine, and allowed it to ferment (a) under anaerobic conditions at 15° C. and at 35° C., and (b) under aerobic conditions at the same temperatures.   The results were:

(1)   2 kilos faeces + 200 c.c. urine left for 2 months.

| | | Difference at end | | | |
| | | Anaerobic conditions | | Aerobic conditions | |
	At start	at 15° C.	at 35° C.*	at 15° C.	at 35° C.
Nitrogen as ammonia	2·27	+ 1·74	+ 1·87	+ 1·65	+ 0·21
„ „ amide	1·74	− 1·47	− 1·66	− 1·45	− 1·66
„ „ other compounds ...	4·55	− 0·42	− 0·22	− 0·42	− 0·26
Total nitrogen	8·56	− 0·15	− 0·01	− 0·22	− 1·46
Loss of nitrogen, original total =100	—	1·7	0·1	2·5	16·8
Gain of ammonia, original total =100	—	20·3	21·8	19·3	2·6

   * In this experiment at 35° C. the initial quantities were not quite the same as in the others.

(2)   2 kilos faeces + 50 c.c. urine diluted to 200 c.c. left 4½ months.

| | | Difference at end | | | |
| | | Anaerobic conditions | | Aerobic conditions | |
	At start	at 15° C.	at 35° C.	at 15° C.	at 35° C.
Nitrogen as ammonia	1·25	+0·82	+0·80	+0·20	+0·01
„ „ amide	0·21	− 0 09	+0·08	−0·02	+0·30
„ „ other compounds ...	5·00	− 0·72	− 0·85	−0·61	−0·81
Total nitrogen	6·46	+0·01	+0·03	−0·43	−0·50
Loss of nitrogen, original total =100	—	+0·2	+0·4	6·7	7·6
Gain of ammonia, original total =100	—	12·7	12·4	3·0	0·1

[1] *Verslag. Landbouwk. Onderzoek. Rijkslandbouwproefstat.* No. 1, p. 21, 1907; No. 7, p. 106, 1910,

In one important respect these results differ from ours: Sjollema and de Wildt found no more ammonia accumulating at 35° C. than at 15° C., while we found notably more at 26° than at 15°.

The results outlined in the preceding paragraphs are precisely similar to those obtained for sewage, and we have already seen that the same statement holds good for the anaerobic decompositions. There is, therefore, a complete parallel between the decompositions of sewage and of manure. Further, there is a close resemblance between these and the laboratory decomposition of protein. We therefore conclude that the decompositions in all cases start in the same way: under strictly anaerobic conditions they remain the same, but under aerobic conditions further reactions, notably formation of nitrate and loss of nitrogen, set in both in sewage and in manure heaps, which mask the general similarity with the degradation of protein as it has been studied in the laboratory.

The proof that the missing nitrogen is evolved as gas.

In all discussions hitherto it has been assumed that the missing nitrogen is evolved as gas, but no definite evidence so far as we know has been adduced in proof. In any experiment on the subject it is obviously necessary that the decomposition should proceed under conditions as nearly as possible like those obtaining in nature, and at the same time so rigidly under control that any change in the volume of gaseous nitrogen can be detected. We have succeeded in carrying out the experiment by keeping farmyard manure in a hermetically sealed apparatus so arranged that the air was kept in constant circulation, fresh oxygen introduced whenever necessary, and CO_2 removed soon after it was formed.

The disposition of the apparatus is shown in Fig. 9. A weighed quantity of farmyard manure of known composition was placed in the flask A, connected to an acid bulb B (to absorb ammonia), an alkali bulb C (to absorb CO_2)[1] and a soda lime tube D, and to the mercury pump E, whereby a stream of air could be drawn through. The pump was so arranged that the whole of the air was delivered back into the flask. The system was closed and it was made rigidly airtight and absolutely beyond any possibility of leakage by building the apparatus up with the aid of the blowpipe and making all the joints of blown glass, and further by having mercury seals on the tap and the flask. A manometer F was attached so that changes in pressure could be read:

[1] The glass spiral between B and C is to relieve the strain on the apparatus.

TO WATER PUMP ←

A   B   C   D   E   F   G   H
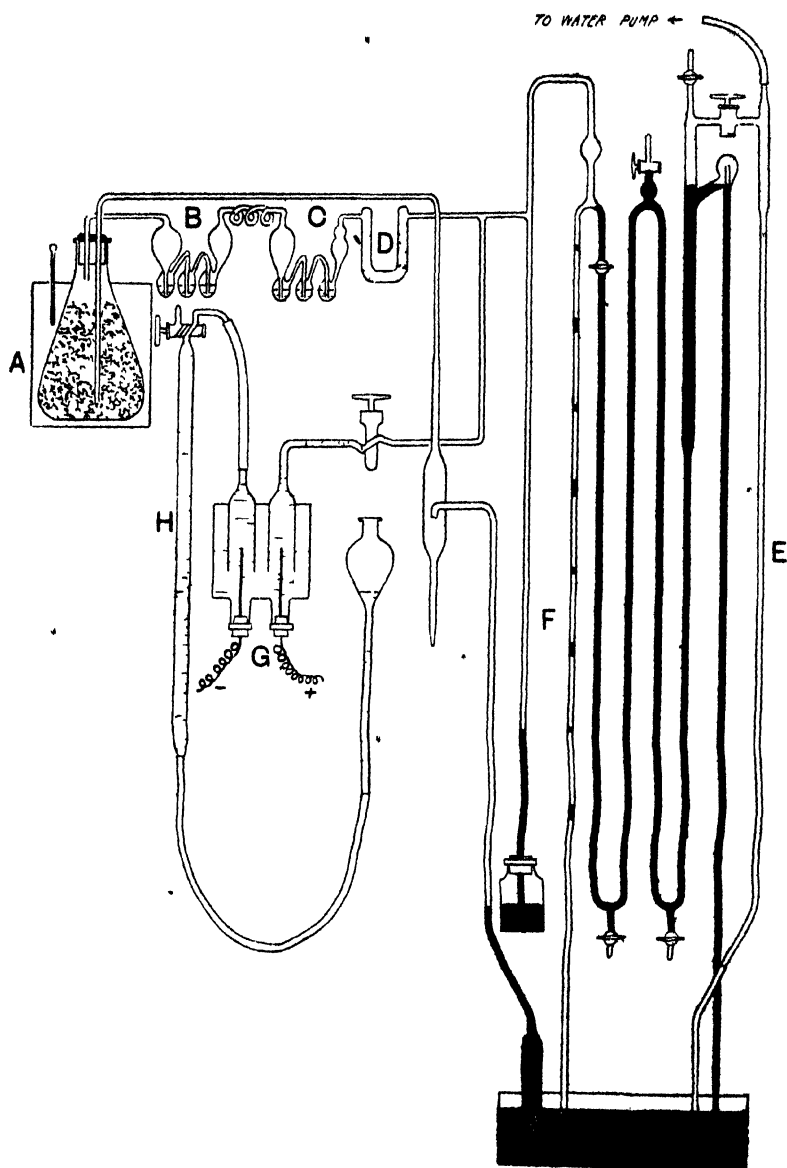
Fig. 9. Apparatus used to ascertain whether the nitrogen lost during the decomposition of farmyard manure is evolved as gas.

any absorption or evolution of gas could thus be measured. As oxygen was absorbed in quantity it was essential that a pure supply should be available, free not only from sulphur dioxide, carbon dioxide, etc., but also from traces of nitrogen: this was ensured by sealing on to the apparatus an electrolytic vessel G, charged with baryta, by the electrolysis of which very pure oxygen was obtained. The volume of oxygen was determined by measuring the volume of hydrogen in the nitrometer H.

The manure having been put into the flask A, and the last joints blown, the pump was started working and sufficient air was extracted to allow the auxiliary mercury lifting pump to come into action. The system was then completely closed, the circulation commenced, and the pressure on the manometer read.

The volume of the apparatus now had to be determined. This was done by letting in a known volume of oxygen from the electrolytic vessel and measuring the drop in pressure thus caused.

Knowing the volume of the apparatus and the percentage composition of the air contained therein, it was easy to calculate the volume of nitrogen initially present.

The circulation was then kept up for several hours a day, the mercury being lifted by the auxiliary water pump so that the operation was automatic. The acid in the one set of bulbs caught any trace of ammonia carried over, while the alkali in the other set took out the CO_2: the movements of the manometer showed the net change in pressure. Fresh oxygen was periodically admitted from the electrolytic vessel.

An occasional sample of gas was pumped out, measured and analysed and the manometer was read. Thus the composition of the air inside the apparatus could be known whenever desired.

When the experiment had continued long enough the gas was pumped out, measured and analysed. This gave the volume of nitrogen finally present.

Lastly the manure was collected from the flask, weighed and analysed, and the acid from the bulbs was also distilled to collect the ammonia, which was added to that in the manure. Thus we know the change in amount of nitrogen in the manure, and by a totally different process the change in volume of the nitrogen present. In our two experiments these two distinct methods gave concordant results.

In the first experiment the changes in the manure (bullock manure) were:

	Percentage composition			Weight of manure grams	Weight in grams of		
	Dry matter	Total Nitrogen	Nitrogen as NH_3		Dry matter	Total Nitrogen	as]
At start ...	27·70	0·777	0·166	627·0	173·7	4·872	1·041
After 31 days	25·55	0·705	0 141	617·1	157·7	4·351	0·870
			Loss, grams	9·9	16·0	0·521	0·171
			Percentage loss		10·7		—

Thus there had been a distinct loss of nitrogen.

The changes in the air in the apparatus were:

	Percentage composition				Pressure in apparatus, mm.	Volume in c.c. reduced to N.T.P.		
	N_2	O_2	CO_2	Combustible gas (CH_4 and H_2)		Total	N_2	Combustible gas
At start ...	79 03	20·97	nil	nil	534·4	989	782	nil
After 31 days	81 0	nil	nil	19·0	598	1136	903	203
					Gain	147	121 c c.	203

Volume of apparatus   1500

Volume of oxygen at start ...   ... 290 c c. at N T P

Volume admitted during experiment   61·5

Total used up   ...   . 351·5 c c.

This had not sufficed to keep the conditions aerobic and at the end the whole of the oxygen had been used up and 203 c.c. of combustible gas produced. There had, however, been a notable evolution of nitrogen gas, amounting to no less than 121 c.c. Thus the experiment shows that evolution of nitrogen occurred under the mixed aerobic and anaerobic conditions obtaining in the experiment.

Although the experiment is quantitative in form it is not quantitative in result. The loss of nitrogen in the manure and the gain of nitrogen in the apparatus do not correspond. It could hardly be expected that they should: both are small differences between large figures all of which are liable to some error.

It will be shown later that the initial and final volumes of nitrogen agreed to within 20 c.c. when analysis of the manure proved that there had been no loss and therefore no evolution of nitrogen: in the experiment now under discussion there had been an increase of 121 c.c., which is therefore far above the experimental error. We may therefore take it that nitrogen is evolved in the gaseous form in the aerobic decomposition of farmyard manure.

The conditions of aeration were probably not unlike those of the experiments described on p. 533: and there is a general similarity in the result. But this form of apparatus allows the attainment of much

* more perfect aerobic conditions than is otherwise possible: by letting in the oxygen in quantities of 80 to 100 c.c. at a time—corresponding to a difference of pressure of 50 to 60 mm.—it can be driven into every part of the manure with much more certainty than when a slow air current-is passed through and diffusion is the only factor at work.

In the second experiment this more complete aeration was attained and the result was entirely different. Decomposition was rapid, and no less than 17·2 grams of dry matter disappeared; quantities of CO_2 were evolved; but there was no loss of nitrogen.

The changes in the manure (bullock manure)[1] were:

	Percentage composition			Weight of	Weight in grams of		
	Dry matter	Total Nitrogen	Nitrogen as NH_3	manure grams	Dry matter	Total Nitrogen	Nitrogen as NH_3
At start ...	24·80	0·504	0 076	502·0	124·5	2·53	0·38
After 20 days ...	21·45	0·525	0·083	500·0	107·3	2·63	0·41
			Loss, grams	2·0	17.2	nil	nil

Nor was there any gain in the nitrogen gas.

The changes in the air of the apparatus were:

	Percentage composition				Pressure in apparatus mm.	Volume in c.c. reduced to N.T.P.			
	N_2	O_2	CO_2	Combustible gas (CH_4 and H_2)		Total	N_2	O_2	Combustible gas and CO_2
At start ...	79·03	20·97	nil	nil	488	648	513	—	nil
After 20 days	97·53	0·53	1·94		426·2	546	533	3	10
					Gain		20 c.c.		

Volume of apparatus 1067 c.c.

Thus the change has been only 20 c.c., too small to be regarded as significant[2]. In this experiment, therefore, the analyses both of the manure and of the gas agree in showing that no evolution of nitrogen occurred.

[1] The manure was fresher and contained more straw than the last sample; its content of nitrogen was lower.

[2] At first sight 20 c.c. may appear to be rather a large experimental error. It is however exceedingly difficult to get an accurate value for the initial and final volumes of air. The apparatus is in effect a volumenometer, and in addition to the difficulties inherent in this form, there is the further difficulty that the tension of aqueous vapour is not constant throughout, but is different in the flask from what it is in the reagent bulbs B or C, or the capacity bulb on the way back from the pump to the flask. These uncertainties cause errors in the initial and final amounts, and therefore still greater errors in the difference used in these calculations. The difference observed in the experiment on p. 538 is six times the error of the experiment.

Total oxygen added	...	...	2231 c.c.
Initially present	...	...	135 ,,
Total...	...	...	2366 ,,
Finally left ...	...	...	3 ,,
Total oxygen used	...	...	2363 ,,
Dry matter lost	...	...	17·2 grms..
Nitrogen evolved	...	...	nil

The important result brought out here is that *no loss of nitrogen occurred under completely aerobic conditions* but only under the mingled aerobic and anaerobic conditions obtaining when air diffuses into the manure.

The following conclusions may be drawn from the experiments described in this section:

1. Under completely anaerobic conditions there is no loss of nitrogen, the sum total of the nitrogen in its various combinations remains unchanged, but there is a breaking down of complex nitrogen compounds to ammonia.

More ammonia accumulates at 26° than at 15°.

2. Under completely aerobic conditions there is also no loss of nitrogen, the sum total of nitrogen in its various combinations remaining unchanged, although vigorous oxidation is proceeding.

3. In the mixed aerobic and anaerobic conditions obtaining when air diffuses into farmyard manure there is a loss of nitrogen which has been traced to an evolution of nitrogen gas. The total amount of decomposition is greater than under anaerobic conditions: it is possible that more ammonia is formed, but if so it does not survive.

4. The transformations of nitrogen compounds in farmyard manure closely resemble those in sewage beds under both aerobic and anaerobic conditions. Under anaerobic conditions they resemble also those of the bacterial decompositions of protein. We suppose, therefore, that the same reactions take place in all three cases. Under partial aerobic conditions the resemblance ceases. It is not necessary, however, to assume any change in the course of the reaction: the facts can all be explained by supposing that in all cases the complex nitrogen compounds break down in the same way to amino-acids and ammonia. But in the case of sewage and farmyard manure further reactions set in, —nitrification and loss of nitrogen,—which mask the resemblance to laboratory decompositions of protein.

Comparison of laboratory experiments with the manure heap experiments.

In comparing the results of the laboratory experiments with those of the manure heap the most striking difference comes out under anaerobic conditions. In the laboratory experiments ammonia accumulates; in the heap it does not.

Under partial aerobic conditions there is complete similarity in the products, but a difference in the extent to which the reactions proceed. The maximum percentage losses have been as follows:

Bullock manure		Dry matter	Total nitrogen	Nitrogen as NH_3	Nitrogen as amide	Nitrogen as other compounds
In laboratory ...	...	17	33	73	75	27
Covered heaps	..	48	42	92	39	32

The difference under anaerobic conditions does not seem to present any great difficulties: it may be explained by supposing that the heap is never wholly anaerobic, but that air always diffuses into it at some period of its history.

We may take it, therefore, that the reactions in the heap are the same as those discussed in the last section. We must now proceed to deal with the reactions which differentiate the decomposition of farmyard manure from that of protein under laboratory conditions, viz. the formation of nitrate and the loss of nitrogen.

The formation of the nitrate in the heap.

Our experiments show that nitrification will take place in the heap, but the accumulation of nitrate depends on two factors,—the presence of air and the absence of much moisture. In the laboratory experiments the manure remained uniformly moist, and nitrate was never observed, in spite of the free access of air. In the heap experiments nitrate was always observed on the outside layers where drying had occurred, but we never found it in the lower depths that had remained moist. Thus, in a heap kept in the open the figures were:

Depth below surface	outer crust $\frac{1}{4}$—$\frac{1}{2}$ inch	1 inch	2 ins.
Nitrate formed, parts of N per cent. ...	0·012	0·006	Trace None

(Bullock dung, Jan. 7, 1915, see pp. 513 and 560)

while in a heap of the same manure kept under cover, which became drier to a much greater depth, the results were:

Depth below surface	outer crust $\frac{1}{4}$—$\frac{1}{2}$ inch	2 ins.	5 ins	10 ins.
Nitrate formed, parts of N per cent.	0·12 0·12	0·12	0·12	0·12

(Woking heap, see pp. 516 and 562).

These results clear up a problem that has caused considerable controversy in the past. Many observers found nitrate in the manure heaps, while others have failed to do so. Probably some of the supposed nitrate was simply an error in manipulation, especially where reduction methods only were employed, but in many cases the result was undoubtedly sound. But some of the negative results were equally sound. Sometimes the discrepancy has been attributed to age, sometimes to other.factors, but we believe that those who found nitrate were dealing with the dry outside of the heap, while those who did not were dealing with the moist inside or with a mixed sample of the whole heap,—outside and inside. Now the nitrate of the outer crust rapidly disappears in contact with moist manure, so that a sample drawn in the ordinary way to represent the heap soon contains little if any nitrate, even if some was originally present. Whether the dryness necessary for the accumulation of nitrate is required simply to protect it against denitrification, or whether it is needed by the nitrifying organisms to insulate them from their neighbours or their neighbours' products, or whether other causes operate, are questions for further investigation.

ON THE CAUSE OF THE EVOLUTION OF NITROGEN DURING THE DECOMPOSITION OF MANURE.

Earlier work.

It has long been known that losses of nitrogen occurred during bacterial decomposition of organic matter which could not be attributed to the volatilisation of ammonia, and which were put down to an evolution of gaseous nitrogen.

The first records we have been able to find go back to the time when the sources of nitrogen for vegetation were being investigated, and attempts were made to set up a balance sheet showing the relation between the amounts of nitrogen in the plant and the soil at the beginning and at the end of the experiment. Reiset[1], Ville[2], and Boussingault[3], who were first to make these experiments, sometimes found less nitrogen in soil + plant at the end of an experiment than in soil + seed at the beginning, and attributed the difference to an evolution of free nitrogen. Some of these early observations were

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[1] Reiset, *Jahresbericht der Chemie*, 1856.

[2] Ville, *Recherches Expérimentales sur la Végétation*, 1857.

[3] Boussingault, *Ann. de Chim. et de Phys.* (III.) 46, 1856, and *Compt. Rend.* 1858, 47 (see his collected works).

probably faulty by reason of the crudeness of the analytical methods, but Lawes, Gilbert and Pugh[1] showed that the losses of nitrogen undoubtedly took place sometimes, though not always, when nitrogenous organic matter,—wheat-meal, barley-meal, or bone-meal,—was made into an "agglutinated condition" with water, and allowed to decompose in presence of air. Practically no ammonia could be detected. Lawes and Gilbert suggested three possible reactions:

1. An oxidation analogous to that of the action of chlorine on ammonia, by which free nitrogen is evolved.

2. A reduction similar to that of a great number of substances upon the oxygen compounds of nitrogen, by which the oxygen is appropriated and the nitrogen set free.

3. These two actions may operate in succession the one to the other.

Little attention was paid to these results at the time. Later on, however, losses of nitrogen were found to occur in the purification of water and of sewage. Angus Smith[2] in 1863 observed an evolution of gaseous nitrogen from a dilute solution of putrefying blood, and showed that nitrates gave off nitrogen under certain circumstances. The earlier sewage workers, Frankland[3] and others, did not actually mention any loss of nitrogen during sewage purification though the published results show that loss took place. Later sewage workers recognised the loss, and Letts[4], indeed, made measurements of the evolved nitrogen, special gasimetric methods being devised for the purpose.

A serious attempt to grapple with the problem was made in 1896 and 1897 at some of the German Experiment Stations, notably Jena, in consequence of the request made by the German Agricultural Society for an investigation into the losses of nitrogen from farmyard manure. These losses were well known to occur, and there was reason to suppose (see p. 504) that they did not arise wholly from volatilisation of ammonia. The first hypothesis, set up by Wagner, was a reduction hypothesis to the effect that nitrates are present in the manure, and these decompose in absence of air giving rise to nitrogen, the change being the one that

[1] Lawes, Gilbert and Pugh, *Phil. Trans.* 1861, 431–577, "On the sources of the nitrogen of vegetation."

[2] Angus Smith, *Memoirs Manchester Lit. and Phil. Soc.* 1865, vol. XXII. (vol. II. of 3rd Series), 47–63; 1867, vol. XXIV. (vol. IV. of 3rd Series), p. 37; also *Report to the Local Govt. Board,* 1882.

[3] Frankland, Denison and Chalmers Morton, *Royal Commission, Pollution of Rivers,* 1868, vols. I.—IV.

[4] *Loc. cit.* (see p. 527 above).

was discovered by Gayon and Dupetit[1] at Nancy, and studied in
Germany by Stutzer[2].

Immendorf[3], on the other hand, favoured an oxidation hypothesis,
and considered that evolution of nitrogen took place in presence of
oxygen, and was the result of direct oxidation or combustion of the
nitrogen compounds to gaseous nitrogen. No reduction of nitrate was
assumed. Pfeiffer and his assistants at Jena[4] began on the reduction
hypothesis and supposed that nitrates were formed on the outside of
the heap, and then diffused inside, where they were denitrified. But
during the course of their experiments they were led to change their
view, and they ended by accepting the oxidation hypothesis. As their
results are typical of much that has been done they may be noticed
in some detail. In the first series of experiments two lots of cow manure
(made with peat moss litter) were put up: air was blown *through* one,
and *over* the other. The losses of nitrogen were:

	grams	per cent.
Air blown through ...	4·26	42·6
Air blown over ...	2·76	27·6

They argue that blowing air *over* the dung would be favourable to
a nitrification and denitrification process, while blowing air *through*
would be *un*favourable to denitrification, since this requires an absence
of air. Yet it causes greater loss.

They also showed that loss of nitrogen went on in experiments
where nitrification was not observed, and further that the effect of
antiseptics on the extent of the loss was not what might be expected
from a knowledge of their effect on denitrifying organisms. These last
two considerations are in the nature of negative arguments and would
not in themselves prove much. The first argument,—that loss is
increased, and not diminished, by blowing air through the manure
instead of over it,—is more positive, but unfortunately it was found
later on to be unsound, for the authors showed that denitrification took
place in their apparatus just as rapidly when air was blowing through
as when it was excluded. They realised that they had not well estab-
lished the case against denitrification, but they still thought that the

[1] *Comptes Rendus*, 1882, **95**, 644–46, and 1365–67: also *Mémoires de la Soc. des Sciences
physiques et naturelles de Bordeaux*, 1886, Séries III. Vol. II.
[2] *Journ. f. Landw.* 1894, p. 383.   [3] *Landw. Jahresbücher*, **21**, 317.
[4] Th. Pfeiffer, E. Franke, C. Götze, H. Thurmann, "Beiträge zur Frage über die bei
der Fäulnis stickstoffhaltiger organischer Substanzen eintretenden Umsetzungen," *Landw.
Versuchs-Stat.* 1897, **48**, 189–245.

oxidation hypothesis fitted the facts better, the ammonia being supposed to be oxidised direct to nitrogen, and not to form nitrate at all. This view was commonly adopted in Germany[1], and it received some support by the announcement in 1897 that Wood and Wilcox[2] had isolated from bran infusions an organism able to liberate nitrogen direct from nitrogenous compounds: so far as we know, however, this has not been confirmed.

On the other hand, Müntz and Lainé[3] working on sewage purification, and dealing with percolation filters, reject the oxidation hypothesis. In the first instance they note that loss only occurs in presence of organic matter: it is not seen when a solution of ammonium salts is run on to the filter. Secondly, they find that the addition of nitrate to the liquor increases the loss of nitrogen. Laboratory experiments showed that it caused an evolution of nitrogen, and by working in a vacuum a complete balance was made as follows:

			At start	At end	
Nitrogen as ammonia	...	...	16·3	15·2	mgms.
Nitrogen in organic compound		...	12·2	13·1	,,
Nitrogen as nitrite	...	...	1·2	0·5	,,
Nitrogen as nitrate	...	...	30·6	8·0	,,
Total	...	...	60·3	36·8	,,
Loss ...	...	...	—	23·5	,,

But they found dissolved in the liquid:

Free nitrogen 38·1 c.c. at N.T.P. = 23·9 mgms.

which thus exactly balances the loss.

This experiment shows that denitrification can take place and cause loss of nitrogen, though it does not necessarily exclude the hypothesis of a direct oxidation of ammonia.

Sjollema and de Wildt, in the investigations referred to on p. 534 also support this alternate nitrification and denitrification view on the grounds that:

1. Loss only occurs under aerobic conditions, and it is only under these conditions that nitrate is formed.

2. No loss occurs, even under aerobic conditions, if the temperature reaches 50° C., the thermal death point of the nitrifying organisms.

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[1] Thus Rumker, *Mitt. Landw. Instit. Breslau*, 1909, 4, 853–872, "Zur Frage der Stall-mistzersetzung," directs attention to the great amount of nitrification that would be required if the whole of the nitrogen were lost by alternate nitrification and denitrification, and asks where the lime is to come from, apparently overlooking the fact that only a small amount is necessary as it could be used over and over again.

[2] *Journ. Soc. Chem. Ind.* 1897, 16, 510.

[3] *Annales Instit. Agron.* 1911, 10. 2nd series, 1–48.

The cause of the loss.

Summing up the work previously done we find that there are three hypotheses in the field to account for the loss of nitrogen during the bacterial decomposition of organic matter:

1. A simple reduction, or denitrification,—nitrates being reduced to gaseous nitrogen.

2. A simple oxidation, the ammonia, or other nitrogen compound, being oxidised direct to nitrogen.

3. An alternate oxidation and reduction, nitrate being formed at one time and reduced at another.

The first hypothesis obviously requires anaerobic conditions: it is excluded by the experiments described on pp. 528–530.

The second hypothesis requires only aerobic conditions: this also is ruled out by the experiment described on p. 539. But in view of its importance it was still further tested, this time with the separate constituents of the manure. Working with urine, with mixtures of urine and straw, and urine and faeces, we obtained vigorous oxidation; in one case as much as 10 % of the nitrogen being changed into nitrate, but the conversions were quantitative and there was no loss of nitrogen during the process.

The quantities used were as a matter of convenience much smaller than in the previous experiments, otherwise the general arrangements were the same. The following are typical of the results obtained with urine:

| | No soil added | | Soil extract added | | Soil added |
	Slow air current (1 litre per hour)	Fast air current (10 litres per hour)	Slow air current	Fast air current	Fast air current
Nitrogen at start	0·0575	0·0575	0·0575	0·0575*	0·0662†
Nitrogen at end	0·0566	0·0567	0·0562	0·0568	0·0647
Loss of nitrogen	0·0009	0·0008	0·0013	0·0007	0·0015
„ „ „ percentage...	1·6	1·4	2·3	1·2	2·3
Nitrate formed, per cent. of total nitrogen	nil	nil	nil	nil	11·1

* In this experiment only 53 % of the ammonia formed was carried over and the liquid was probably too alkaline for nitrification to occur.

† In this experiment 82·% of the ammonia was carried over and the liquid was therefore less alkaline: hence nitrification took place.

A similar result was obtained with a mixture of urine and straw, and of urine and faeces. In an experiment lasting for 34 days in a

fast air current, a little soil being added in each case to ensure a good infection, and the temperature being maintained at 26° C., the results were:

				Liquid urine alone	Urine on glass wool	Urine on straw	Urine + faeces + straw		
Nitrogen at start	...	...	...	0·0179	0·0179	0·0246	0·0246	0·0331	0·0315
Nitrogen at end	..	...	...	0·0178	0·0178	0·0253	0·0243	0·0332	0·0313
Loss of nitrogen	...	...	...	0·0001	0·0001	+ ·0007	0·0003	+ ·0001	0·0002
„ „ percentage			...	nil	nil	+3·1	1·3	nil	nil
Nitrate formed, per cent. of total nitrogen ...	...	...	...	17	nil	nil	nil	nil	nil
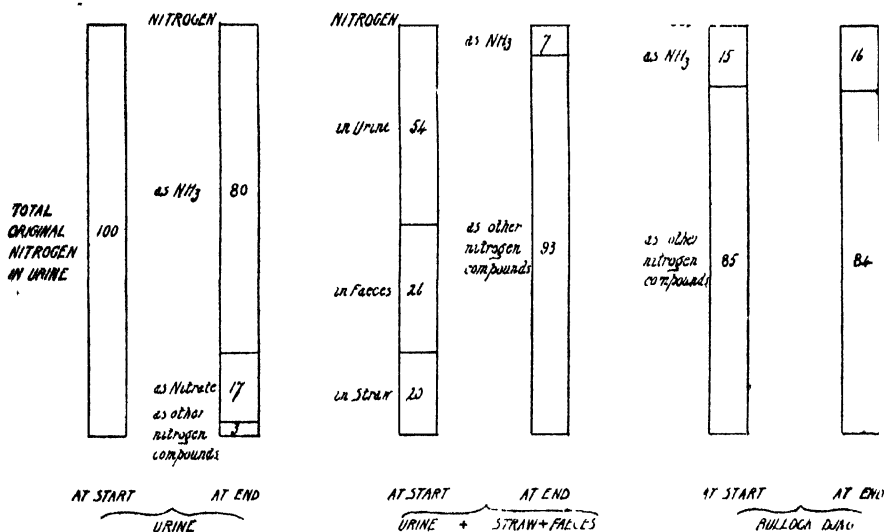
Fig 10. Recovery of nitrogen under good aerobi · conditions from (1) fermenting constituents of farmyard manure artificially mixed, (2) bullock manure naturally made.

As much as 17 % of the total nitrogen was nitrified in the first of these experiments, but there was no loss of nitrogen (Fig. 10).

These experiments show that no loss of nitrogen occurs from urine, straw or faeces, singly or mixed, under completely aerobic conditions: we have already shown (p. 539) that it does not take place from dung. The result is in agreement with Adeney's work[1] on the decomposition of albuminose, asparagine, etc., in dilute solutions saturated with oxygen, where also no loss of nitrogen occurred.   A simple oxidation hypothesis is therefore ruled out.

[1] Adeney, Proc. Royal Irish Acad 1905; also Royal Commission on Sewage Disposal. 5th Report, Appendix VI. 1908, pp. 13–20.

We are left therefore with the fact that loss of nitrogen only occurs under the partial aerobic and anaerobic conditions that obtain when the mixed trampled materials of farmyard manure are exposed to air; it will not go on in simple aerobic or anaerobic conditions. This complex air requirement indicates an alternate oxidation and reduction, as postulated by the third hypothesis.

The simplest possibility is that nitrates are formed in one part of the heap, and decomposed in another. We have shown that nitrification can set in in a manure heap when air is present and the moisture has been reduced below a certain critical point. Further we have shown that when this nitrate reaches the moister part of the heap it speedily denitrifies. Whenever, therefore, nitrate can form, and can get into the interior, there is the possibility of the loss of nitrogen. We have never found nitrate in the wet part of the heap, or we might suppose that it is formed when the current of air happens to travel that way, and decomposed when the air passes elsewhere. Our failure to find nitrate does not necessarily mean that none is formed there, but only that nitrification, if it occurs, is slower than denitrification.

It is not necessary, however, that the oxidation should proceed as far as the nitrate stage: any intermediate stage would suit the hypothesis equally well.

Whatever the actual compound formed, this hypothesis requires three stages for the loss of nitrogen:

1. Ammonia is formed. This is necessary because so far as is known no compound of nitrogen except ammonia is oxidised by bacteria to give the compound postulated for the next steps.

2. The ammonia is oxidised to form an oxygen compound of nitrogen.

3. This compound is reduced, with liberation of gaseous nitrogen.

At first sight it may appear cumbersome that the ammonia should first be oxidised to an oxygen compound, and then reduced: why cannot it be oxidised straight away to gaseous nitrogen? We do not consider that this objection has any force. The oxidation of methane, even during combustion, is known to proceed by the introduction of successive oxygen atoms:

$$
\begin{array}{c}
\text{H} \\
\text{H} \qquad \text{H} \qquad \text{H} \qquad \text{O} \\
| \qquad\qquad | \qquad\qquad | \qquad\qquad | \\
\text{H—C—H} \rightarrow \text{H—C—O—H} \rightarrow \text{H—C}{=}\text{O} \rightarrow \text{H—O—C}{=}\text{O} \rightarrow \text{O}{=}\text{C}{=}\text{O} \\
| \qquad\qquad | \\
\text{H} \qquad\qquad \text{O} \\
\qquad\qquad | \\
\qquad\qquad \text{H}
\end{array}
$$
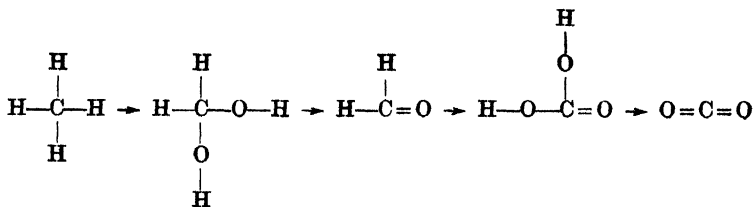
and it is therefore not difficult to suppose that ammonia would oxidise on the same model:

$$\underset{\overset{|}{H}}{\overset{H}{H-N-H}} \rightarrow \underset{\overset{|}{H}}{\overset{H}{H-N-O-H}} \rightarrow \underset{\overset{\|}{O}}{\overset{H}{N-O-H}} \rightarrow \underset{\overset{\|}{O}}{O-N-O-H}$$
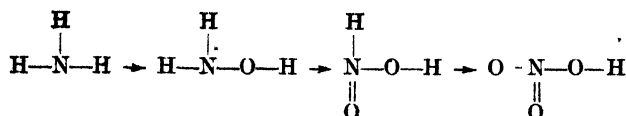
It is known also that nitrates and nitrites are rapidly reduced to gaseous nitrogen under anaerobic conditions.

This hypothesis is consistent with the general results obtained by sewage workers who, as already stated, have accumulated a vast amount of data on the subject. They have shown that there is no loss of nitrogen under anaerobic conditions,—that it only goes on under aerobic conditions, but that beyond a certain point excess of air reduces the loss. Thus, on the contact bed the conditions, though aerobic, are less so than on the percolating filter, and the loss is greater: the higher amount of dissolved oxygen on the percolating filter reducing the loss. Similarly when the dissolved oxygen is increased in any other way (e.g. by adding tap-water), a reduction of the loss occurs (see p. 527).

Thus the alternate nitrification and denitrification hypothesis will fit all the facts. But it is by no means definitely established, and it suffers from a rather serious drawback: it involves either a transfer of nitrate from the exterior to the interior of the heap in conditions where actual movement is difficult to imagine (e.g. in heaps under cover or manure stored in bottles), or a formation of nitrate in the interior of the heap where we have never yet been able to observe it. We therefore prefer another hypothesis which equally fits the facts and has the advantages of freedom from any mechanical difficulty, and of being the more general hypothesis of which alternate nitrification and denitrification is only a particular case.

We have already seen (p. 524) that under anaerobic conditions molecular groupings tend to arise, which become unstable as soon as aerobic conditions set in, and vice versa. These changes have usually been studied in non-nitrogenous compounds, but it is reasonable to suppose that nitrogen compounds may be similarly affected also. If, for example, a derivative of propionic acid formed under anaerobic conditions can on entry of air shorten its chain by eliminating —CH_2—, and becoming a derivative of acetic acid, it is at least likely that some complex nitrogen compound could simplify itself by eliminating nitrogen.

Assuming the possibility of such a change the mechanical conditions present no difficulty. Where air penetrates into the heap the conditions

are aerobic.   As soon as the oxygen is used up in any particular portion, the conditions become anaerobic, and compounds formed under the previous aerobic conditions may decompose.   When, owing to change of temperature, or other conditions, more air diffuses into this portion, the conditions again become aerobic, and compounds formed under the anaerobic conditions may decompose.   Thus we have a perpetual succession of decompositions so long as the conditions fluctuate between aerobic and anaerobic

It will be seen that nitrification and denitrification form a particular case of this hypothesis.   We prefer the general form because other reactions of the sort are by no means out of the question.

The conversion of ammonia into complex nitrogen compounds.

Reference has already been made (p. 504) to the claim set up by Maercker and Schneidewind that the amount of quick-acting nitrogen (i.e. ammonia and amide) in the manure is considerably less than the digestible nitrogen in the food, while conversely the amount of slow-acting nitrogen in the manure is much greater than that of the indigestible nitrogen of the food.   Some of their results are:

	Indigestible N in food, litter, etc. grams	N as other nitrogen compounds in dung grams	Gain grams	per cent.
Well trodden dung (deep stall) ...	120·9	265·6	144·7	12
Dung kept in covered shed ...	120 9	205·7	84·8	70
„ „ the open	120·9	227 8	106·9	88

They therefore supposed that a considerable conversion of ammonia and amide into complex nitrogen took place during the making of the manure, and they attributed this to microorganisms.

Unfortunately their figures are not entirely experimental, the values for the digestibility of the foods, litter, etc., being assumed, and not determined directly.   Russell and Goodwin[1] were unable to obtain such high numbers.   Assuming mean values for the digestibility of the food stuffs their results came out much lower:

	Indigestible N, in food, litter, etc. grams	N as other nitrogen compounds in dung grams	Gain grams	per cent.
Bullocks fed on poor oil cake ...	7485	9480	1995	21
„ „ rich oil cake ...	6712	7668	956.	14

[1] *Journ. South-Eastern Agric. Coll. Wye,* 1905, No. 14, 187.

The analytical difficulties are considerable, and as the digestibilities are only assumed and not determined, one must not lay too much stress on small differences.

We have made experiments in the laboratory by passing a current of air through the mixture of straw, faeces, and urine for a period of four weeks. Prior to the experiment the urine had undergone a certain amount of fermentation whereby most of its nitrogen had been converted into ammonia. After this experiment the ammonia in the whole-mass was determined: in no case was it equal to the amount present at the beginning, showing that a considerable amount had been converted into more complex nitrogen compounds. The results are given in Table VI.

TABLE VI. *Change of ammoniacal nitrogen into "other" forms of nitrogen during the fermentation of urine, faeces, and straw under aerobic conditions.*

| | Horse urine and faeces | | | | Horse urine and straw | | Horse urine, straw and faeces | |
| | Expt. 1 | | Expt. 2 | | | | | |
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Nitrogen as NH$_3$ ...	30·4	2·4	20·0	7·1	21·3	1·6	18·5	0·0
„ „ other compounds	69·6	98·0	80·0	88·8	78·7	96·5	81·5	111·4
Total nitrogen ...	100·0	100·4	100·0	95·9	100·0	98·1	100·0	111·4

| | Bullock urine and straw | | | Bullock urine and faeces | | | |
| | | | | Expt. 1 | | Expt. 2 | |
	Initial	Final (a)	(b)	Initial	Final	Initial	Final
Nitrogen as NH$_3$	67·3	22·8	13·4	28·1	5·3	26·3	9·3
„ „ as other compounds	32·7	73·5	86·5	71·9	86·2	73·7	88·0
Total nitrogen	100·0	96·3	99·9	100·0	91·5	100·0	97·3

But when we came to deal with masses of farmyard manure, whether we used only the 10 lbs. quantities taken for the laboratory experiments, or the one or two ton heaps used for outdoor experiments, we were unable to find evidence of such a change. In some 20 different experiments only one showed any sign of gain in complex nitrogen compounds, and there the increase was only 8 % on the quantity measured, which in turn was a difference involving three separate measurements.

Only when the heap is composed *entirely* of horse manure do we get any increase in the complex nitrogen compounds. The results are given in Table VII[1].

[1] For further experiments with horse manure see this *Journal*, 1917, **8**. 299.

TABLE VII. *Changes in nitrogen compounds in manure heaps, horse dung, Rothamsted, showing gains of complex nitrogen compounds and loss of ammonia.*

	In the open					
	Loose heap on soil			Loose heap on concrete		
	At start (Jan. 23, 1914)	At end (April 30, 1914)	Per-centage change	At start (Jan. 23, 1914)	At end (April 30, 1914)	Percentage change
Manure in heap ...	1016	741	− 27·1	508	451	− 11·2
Dry matter	254	183	− 28·0	128	90	− 29·7
Ammoniacal nitrogen	0·98	0·10	− 19·2	0·49	0·06	− 19·5
Amide nitrogen ...	0·41	0·44	+ 0·7*	0·19	0·21	+ 0·9*
Other nitrogen ...	3·19	·3·65	+10·0*	1·52	1·72	+ 9·1*
Total nitrogen ...	4·58	4·19	− 8·5	2·20	1·99	− 9·5

.* Gains.

Apart from this special case of pure horse manure we find no evidence that the complex nitrogen compounds tend to increase at the expense of the ammonia *in the manure heap.* The action is quite possible, indeed it is brought about in the laboratory experiments, but the conditions of the heap do not seem to favour it. We cannot help thinking that there is some mistake in Maercker and Schneidewind's figures, and we are strengthened in this view by the circumstance that no other investigator of repute has, so far as we know, obtained anything like such high results.

SUMMARY OF RESULTS, AND APPLICATION TO THE STORAGE OF MANURE.

We can now bring together the various results obtained during the course of our investigations.

We find that the changes are at a minimum under *anaerobic* conditions, and they are as follows:

1. In the laboratory experiments as much as 17 % of the dry matter may be converted into gas: in the heap the proportion is less.

2. The non-nitrogenous constituents are particularly affected, as much as one quarter of the pentosans may disappear during the process and other constituents break down in like proportion. The gas evolved contains carbon dioxide, marsh gas, and hydrogen.

3. In the laboratory experiments the nitrogenous compounds also break down, part of the complex compounds giving rise to ammonia. More ammonia is found at 26° C. than at 15° C.

4. No nitrates are formed.

5. In the only heap where we were satisfied that the conditions were anaerobic there was no accumulation of ammonia.

6. There is no loss of nitrogen during the process: the whole of the initial nitrogen being recovered within the error of the experiment.

The *aerobic* changes are as follows:

1. The loss of dry matter is greater and the temperature rises higher than under anaerobic conditions. The gases evolved contain no hydrogen or marsh gas.

2. The loss of dry matter shows some relationship to the aggregate rise of temperature.

3. There is almost always a larger decomposition of complex nitrogen compounds than under anaerobic conditions. There is not usually an accumulation of ammonia in the laboratory, and in the heap there is invariably a loss. $+N H_3$.

4. Nitrate is found in the dry outer portions of the heap, but not in the moister interior, nor was it found in the laboratory experiments where the manure remained moist: the necessary conditions appear to be dryness and sufficient air.

5. Under ordinary conditions of incomplete aeration there is an evolution of gaseous nitrogen, though this is not observed when the conditions are wholly aerobic without any local anaerobic action.

6. The loss of ammonia shows some relationship to the maximum temperature attained.

7. The loss of dry matter is greater from exposed heaps than from sheltered heaps and so is the loss of ammonia, unless this is already at a minimum; but the loss of total nitrogen is not always greater.

8. The loss of nitrogen is not wholly dependent on that of dry matter, since as already stated it does not occur under purely aerobic or purely anaerobic conditions, although other constituents are lost. But the loss of nitrogen that occurs in the mixed aerobic and anaerobic conditions occurring in practice varies under comparable conditions, with the loss of dry matter, all constituents of the heap apparently breaking down simultaneously. An exception occurs when the temperature has risen high, e.g. to 70° C. after which decomposition of nitrogen compounds and loss of nitrogen proceed more slowly than loss of dry matter: an actual concentration of nitrogen in the heap therefore occurs. A second exception is seen in exposed heaps, when the loss of dry matter is usually proportionally greater than that of nitrogen.

9. The loss of nitrogen might occur by

(a) washing away of soluble nitrogen compounds,

(b) volatilisation of ammonia,

(c) evolution of nitrogen,

(d) other ways.

From the sheltered heap (a) is excluded.

It is further shown that (b) can hardly account for the observed losses in the heap, and certainly not for those in the laboratory experiments, where the extent of volatilisation was measured, and found to be only small. In the laboratory experiment an evolution of nitrogen has been demonstrated and presumably a similar change goes on in the heap.

10. In the laboratory experiments decomposition never proceeded very far, the maximum losses being 17 % of dry matter, 30 % of complex nitrogen·compounds, and 33 % of total nitrogen.

11. In our heap experiments we find this last fraction of complex nitrogen compounds, representing 50 to 60 % of the original total nitrogen, only decomposes very slowly indeed.

We can now come to the application of these results to the practical problem of storing farmyard manure. It is unnecessary for us to go into detail as this has already been done elsewhere[1]. We may, however, indicate the general outlines. In the first instance we must be quite clear as to what is wanted. This can only be determined by field trials, and our experiments recorded on pp. 505–507 show that the main factors determining the value of farmyard manure are the total nitrogen and the ammonia: indeed it is possible to evaluate the manures on this basis provided the ammonia figures are adequately "weighted." The total dry matter is of obvious importance in view of physical effects, but we have no information as to the value of any particular· non-nitrogenous constituent. Sjollema and de Wildt regarded the pentosans as undesirable on the theoretical ground that they favour denitrification, but there is no sufficient evidence that the loss of nitrogen would be less without them.

The objects to aim at in a manure heap must be to secure

(1) as much dry matter,

(2) as much ammonia, and

(3) as little loss of nitrogen as possible.

Now the laboratory experiments show that these objects can all be attained by storing the manure heaps under anaerobic conditions at about 26° C. There is a considerable formation of ammonia and no loss of nitrogen, although some loss of dry matter occurs.

[1] *Journ. Roy. Agric. Soc.* 1917, **77**, 1–35.

The heap experiments, on the other hand, show that these desirable results are not attained in manure heaps, however well put up. However compact the heap some nitrogen is always lost and there is never an accumulation, but commonly a loss, of ammonia. This result is not peculiar to our heaps: it has been obtained by all other investigators whose papers we have been able to find. We conclude, then, that the heap is not the ideal method of storing manure.

The conditions to be aimed at are complete anaerobic conditions and a temperature of 26° C. and it is obvious that these can best be attained in a watertight pit or tank that could be closed so as to keep out oxygen and to keep in the carbon dioxide produced by fermentation. This would be the ideal method for storing farmyard manure.

But as this ideal method presents certain practical difficulties we must see how far the best methods of practice approximate to it, and whether any further improvements can be suggested.

Two cases arise:

1. Manure that can be left undisturbed under the beasts, e.g. manure made in covered yards or stalls by fatting beasts.

2. Manure that has to be thrown out daily, e.g. manure made from dairy stock or from the horse stables.

1. All experiments show that manure left under the beasts suffers a loss of about 15 % of its nitrogen; there is no accumulation of ammonia, but, on the contrary, less ammonia than corresponds with the digestible nitrogen in the food.

This method, therefore, is far from being perfect; but in comparative experiments it has always come out better than heaps, and if the buildings are good and the manure is well made there is probably little scope for further improvement.

Further losses set in as soon as the beasts are removed or the manure is hauled out into a clamp. We have been unable to reproduce the strict anaerobic results in a heap, however well it was compacted, and in only one case,—the compact cow manure heap,—were we even partially successful. There we lost only 4 % of the dry matter, and none of the nitrogen, but we had no accumulation of ammonia. The other compacted heaps showed all the aerobic actions in different degree, in particular there was always a loss of nitrogen.

The losses become more serious if the heap is not properly compacted or if it is left exposed to the weather.

By omitting to compact the heap we lost an additional 3 % of dry matter, and 24 % of ammonia; but in two cases out of three there

was no change in total nitrogen as compared with compacted heaps. In general the effect of compacting is to delay rather than prevent decomposition. By leaving it exposed to the weather we lost an additional 8 % of dry matter, 33 % of ammonia in two experiments and 10 % in another, and 21 % of total nitrogen in two experiments, and 2 % in two others, as compared with sheltered heaps. Field experiments showed that the crop producing power of the manure was considerably lowered by exposure and that even a slight shelter was beneficial[1].

It appears then that the heap is at best an imperfect method of storage, but that its defects are lessened by keeping it compact and sheltered, where it will neither be washed by rain nor suffer too much loss on drying, and in particular by avoiding summer storage.

It is difficult to suggest any alteration in method of clamping that would be a further improvement, the best practical men have probably developed the method as far as its fundamental imperfections allow, and the only hope of further advancement seems to be in the wholly fresh direction we suggest,—to set up some pit or tank where absolute anaerobic conditions can be attained, and where the temperature can be kept at 26° C. or thereabouts. This has been successfully done for silage, the old stack silo having given place to the air-tight concrete structure, and it may yet be accomplished for manure.

2. Manure thrown out daily. From the start the conditions here are aerobic, which, as we have seen, involve marked losses of dry matter, of ammonia, and to a less extent of total nitrogen as well, and these are aggravated when the heaps are thrown out in the open and exposed to the washing of the rain and the drying effects of the sun.

Much can be done to improve matters by carrying the manure into a shelter, such as the Cheshire dungstead, a structure like a small Dutch barn surrounded on three sides by a low wall, the fourth being open to allow the cart to back in, and compact the manure. The Oxford manure house does not allow the manure to be so readily compacted. It is probable that the dungstead could be improved in some details, but at the best it still retains some of the imperfections of the clamp.

We think the best prospect of dealing with manure from dairy cows is to aim straight away at storage in a pit or tank; but as no practicable

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[1] *Journ. Roy. Agric. Soc.* 1917, **77**, 1–35.

device has been worked out[1] for the whole of the manure we are making a commencement with the most valuable part, the liquid excretions. A modification of the Belgian liquid manure tank, which is built under the animal to receive the liquid, furnishes a suitable starting point. These new experiments are being carried out by one of us on the farm of the Hon. Rupert Guinness at Hoebridge.

The solid excretions and the litter would still require to be dealt with, but even if no advance on present methods were made with these the loss would be shorn of most of its seriousness once the liquid were better treated.

The practical conclusions that we draw are:

(a) The method of leaving manure under the beasts in boxes or covered yards until it is wanted remains the best that we can suggest where it is practicable.

(b) If the manure has to be stored it should be under anaerobic conditions, and if possible at a temperature of about 26° C.

(c) No heap, however well compacted or sheltered, fully satisfied these requirements. Probably the making of the heap has been developed to as perfect a pitch as possible, and we have no further improvement to suggest.

(d) The best hope for improvement lies in storing the manure in watertight tanks or pits so made that they can be completely closed, and thereby allow the attainment of perfect anaerobic conditions. The proper temperature would have to be maintained.

We are hoping the experience gained in the new Woking experiments will indicate a method whereby this end can be achieved in practice.

EXPERIMENTAL DETAILS.

1. *Method of taking samples of dung for analysis.* The dung from the bulk heap of fresh manure was thrown, a forkful at a time, on to a sheet of tarpaulin or a wood or concrete floor, and then transferred to the crate to be weighed before stacking in the experimental heaps. A small portion was picked by hand from each forkful and thrown alternately on to two small sample heaps. These small heaps were then subsampled in exactly the same way and each one finally yielded about 20 lbs. of dung which was tightly filled into tins for analysis in the laboratory. In order to obtain more homogeneous

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[1] It is difficult to suggest anything wholly new in agriculture. An illustration of a manure pit can be seen in Hale's *Compleat body of Husbandry*, 1756, and another in an interesting pamphlet by Boussingault, *La Fosse à Fumier*, Paris, Béchet Jeune, 1858.

material for the actual determinations, these final samples were passed through a large meat mincing machine and about one-tenth of the product taken for the working sample in each case.

It is important to note that the whole of the dung was treated in this manner so that a really representative sample should be obtained. From six to fifteen tons were thus handled in each experiment.

2. *The analytical methods.* The laboratory analyses were all made in duplicate. Thus for each item four values were obtained, two from each of the separate samples drawn as described above. Table VIII shows that a satisfactory degree of agreement can be attained.

The nitrogen was determined by the Kjeldahl-Gunning method.

The ammonia was estimated as follows: 10 grams of the manure were added to 500 c.c. of distilled water and sufficient magnesia (about 3 grams) to make the whole alkaline to litmus. It was then distilled at ordinary pressure. This method is quicker than distillation *in vacuo* and while it does not give absolutely sharp results it works sufficiently well. The end point is sufficiently sharp when tested by titration: it is not, however, sharp to the Nessler test.

The amide nitrogen was estimated by digesting 10 grams of the manure with 200 c.c. of 10 % sulphuric acid for 10 hours on the water bath, making just alkaline with caustic soda and distilling. The ammonia figure has to be deducted from the value thus obtained.

The "other nitrogen compounds" are arrived at by difference.

The nitrate was estimated by the zinc-couple reduction method when sufficient was present, but small quantities were approximately estimated by the phenol sulphonic acid method. For qualitative testing, Letts' diphenylbenzidine method gave good results.

3. *The average composition of farmyard manure.* Although it is obviously impossible to speak definitely of an average composition of so variable a material as farmyard manure its composition as made on different farms differs less than might be expected. So much of it is litter, and so little does the indigestible material of the solid faeces vary, that the only notable cause of variation is the urine. The results of some of the most complete analyses are given in Table X.

TABLE VIII. *Showing range of variation in composition between the two samples drawn as described on pp.* 557–58.

Rothamsted Manure Heaps, 1915–16.

Cow dung from a neighbouring dairy farm.

Series G.

	Ammoniacal Nitrogen		Amide Nitrogen		Total Nitrogen		Dry Matter	Ash
Heap No. 1 Loose covered	0·076	0·078	0·026	0·024	0·423	0·426	20·00	4·28
Sample A	0·079		0·022		0·428			
Sample B	0·079	0·079	0·033	0·032	0·398	0·404	19·05	4·25
	0·079		0·031		0·410			
Heap No. 2 Compact covered	0·102	0·102	0·025	0·026	0·422	0·423	19·25	3·70
Sample A	0·102		0·026		0·424			
Sample B	0·098	0·099	0·030	0·031	0·406	0·406	18·60	3·53
	0·099		0·031		0·406			
Heaps Nos. 3 and 4	0·091	0·090	0·026	0·028	0·392	0·395	18·85	3·83
Sample A	0·088		0·029		0·398			
Sample B	0·098	0·097	0·029	0·029	0·425	0·425	18·20	3·60
	0·096		0·029		0·425			
Heap No. 5 Loose heap	0·081	0·083	0·024	0·025	0·404	0·407	18·20	3·64
(Iron roof) A	0·084		0·025		0·409			
Sample B	0·076	0·078	0·024	0·025	0·403	0·403	17·80	3·66
	0·079		0·025					
Average of all	—	0·088	—	0·028	—	0·422	18·74	3·81

All these heaps were made from the same lot of dung which had gradually accumulated in the yard.

TABLE IX. *Composition of manure and summary of changes suffered on storage.*

Cow manure

	Compact and under cover — Readily compacted		Same manure. Re-made on April 30, 1914 — Compacted incompletely		Loose under cover — Moderately aerobic		Same manure heap. Re-made on April 30, 1914 — Increasingly aerobic	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
General character of heap								
Composition								
Moisture	80·9	78·7	78·7	75·8	81·5	79·3	79·3	78·9
Dry matter	19·1	21·3	21·3	24·2	18·5	20·7	20·7	21·1
Nitrogen as NH₃	0·068	0·063	0·063	0·030	0·067	0·044	0·044	0·013
„ „ amide	0·032	0·037	0·037	0·048	0·036	0·045	0·045	0·074
„ „ other comps.	0·254	0·314	0·314	0·366	0·262	0·320	0·320	0·307
Total nitrogen	0·354	0·414	0·414	0·444	0·365	0·409	0·409	0·394
Weight of heap, kilos.	1524	1306	1288	1046	1270	1051	1033	763
Period of storage	Jan. 23—April 30, 1914		April 30—July 21. 1914		Jan. 23—April 30, 1914		April 30—July 21. 1914	

Loss

	On initial quantity	Total N=100	On initial quantity	Total N=100	On initial quantity	Total N=100	On initial quantity	Total N=100
Dry matter, per cent.	4·3		9·0		7·4		26·1	
Nitrogen as NH₃	20·6	4·1	62·2	9·5	45·8	8·4	78·3	8·4
„ „ amide	1·0	0·2	+4·2	+0·4	+3·4	+0·2	+19·2	+2·1
„ „ other comps.	+23·6	+4·3	6·4	4·8	+0·9	+0·7	30·1	23·5
Total nitrogen	nil	nil	13·9	13·9	7·5	7·5	29·8	29·8
Temperature attained	9·0° C.		22·8° C.		16·5° C.		32·0° C.	

Bullock manure

	Compact and under cover — Compacted incompletely		Same manure, but kept for nine months — Compacted incompletely		Loose under cover — Open strawy texture		Same manure, but kept for nine months	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
General character of heap								
Composition								
Moisture	75·4	77·8	75·4	78·6	76·8	76·6	76·8	74·0
Dry matter	24·6	22·2	24·6	21·4	23·2	23·4	23·2	26·0
Nitrogen as NH₃	0·107	0·063	0·107	0·040	0·100	0·035	0·100	0·015
„ „ amide	0·044	0·051	0·044	0·044	0·032	0·064	0·032	0·058
„ „ other comps.	0·394	0·409	0·394	0·439	0·358	0·478	0·358	0·601
Total nitrogen	0·545	0·523	0·545	0·523	0·490	0·587	0·490	0·674
Weight of heap, kilos.	1270	980	1270	768	1016	654	1016	500
Period of storage	Jan. 7—April 14. 1915		Jan. 7—Oct. 11, 1915		Jan. 7—April 14, 1915		Jan. 7—Oct. 11, 1915	

Loss

	On initial quantity	Reckoned on Total N=100	On initial quantity	Total N=100	On initial quantity	Total N=100	On initial quantity	Total N=100
Dry matter, per cent.	30·3		47·5		34·9		44·8	
Nitrogen as NH₃	54·5	10·7	77·3	15·2	77·5	15·9	92·4	18·9
„ „ amide	10·6	0·9	39·3	·3·2	+8·6	+0·4	12·1	0·7
„ „ other comps.	24·9	14·3	32·6	23·6	13·8	10·0	17·4	12·7
Total nitrogen	25·9	25·9	42·0	42·0	25·5	25·5	32·3	32·3
Temperature attained	51° C.		51° C.		71° C.		71° C.	

Cow manure — Loose in open (Moderately aerobic)

	Initial	Final
Moisture	81·9	81·9
Dry matter	18·1	18·1
Nitrogen as NH$_3$	0·068	0·017
", amide	0·028	0·032
", other comps.	0·271	0·298
Total nitrogen	0·367	0·347
Weight of heap, kilos	1524	1216
Period of storage	Jan. 23—April 30, 1914	
Loss	On initial quantity	Total N=100
Dry matter per cent.	80·0	14·8
Nitrogen as NH$_3$	8·8	0·7
", other comps.	12·1	9·1
Total nitrogen	24·6	24·6
Temperature attained	21° C.	

Bullock manure — Loose in open (Open strawy texture)

	Initial	Final
Moisture	77·3	81·6
Dry matter	22·7	18·4
Nitrogen as NH$_3$	0·078	0·012
", amide	0·036	0·048
", other comps.	0·326	0·377
Total nitrogen	0·440	0·437
Weight of heap, kilos	1016	742
Period of storage	Jan. 7—April 14, 1915	
Loss	On initial quantity	Total N=100
Dry matter per cent.	88·7	15·7
Nitrogen as NH$_3$	2·5	0·2
", other comps.	15·4	11·5
Total nitrogen	27·4	27·4
Temperature attained	55° C.	

Same manure heap — Re-made on April 30, 1914 (Increasingly aerobic)

	Initial	Final
Moisture	81·9	78·0
Dry matter	18·1	22·0
Nitrogen as NH$_3$	0·017	0·009
", amide	0·032	0·057
", other comps.	0·298	0·374
Total nitrogen	0·347	0·440
Weight of heap, kilos	1198	832
Period of storage	April 30—July 21, 1914	
Loss	On initial quantity	Total N=100
Dry matter per cent.	66·7	3·3
Nitrogen as NH$_3$	+20·5	+1·9
", other comps.	13·8	11·9
Total nitrogen	13·3	13·3
Temperature attained	29° C.	

Same manure, but kept for nine months

	Initial	Final
Moisture	77·3	75·5
Dry matter	22·7	24·5
Nitrogen as NH$_3$	0·078	0·012
", amide	0·036	0·051
", other comps.	0·326	0·517
Total nitrogen	0·440	0·580
Weight of heap, kilos	1016	376
Period of storage	Jan. 7—Oct. 11, 1915	
Loss	On initial quantity	Total N=100
Dry matter per cent.	94·3	16·8
Nitrogen as NH$_3$	48·6	4·0
", other comps.	41·4	30·5
Total nitrogen	51·3	51·3
Temperature attained	55° C.	

Cow manure — Loose under cover, Watered daily (Moderately aerobic)

	Initial	Final
Moisture	80·9	79·9
Dry matter	19·1	20·1
Nitrogen as NH$_3$	0·046	0·044
", amide	0·028	0·034
", other comps.	0·275	0·276
Total nitrogen	0·369	0·354
Weight of heap, kilos	1270	1144
Period of storage	Jan. 23—April 30, 1914	
Loss	On initial quantity	Reckoned on Total N=100
Dry matter per cent.	40·5	7·3
Nitrogen as NH$_3$	*8·3	+0·6
", other comps.	9·5	6·9
Total nitrogen	13·6	13·6
Temperature attained	15·5° C.	

The same, re-made on April 30, 1914, and watered daily (Moderately aerobic)

	Initial	Final
Moisture	79·9	78·0
Dry matter	20·1	21·1
Nitrogen as NH$_3$	0·044	0·012
", amide	0·034	0·048
", other comps.	0·276	0·302
Total nitrogen	0·354	0·362
Weight of heap, kilos	1126	957
Period of storage	April 30—July 21, 1914	
Loss	On initial quantity	Reckoned on Total N=100
Dry matter per cent.	78·0	9·6
Nitrogen as NH$_3$	+17·9	+1·7
", other comps.	8·5	6·7
Total nitrogen	14·6	14·6
Temperature attained	22° C.	

Bullock manure — Compact in open (Compacted incompletely)

	Initial	Final
Moisture	77·52	83·52
Dry matter	22·48	16·48
Nitrogen as NH$_3$	0·079	0·012
", amide	0·031	0·038
", other comps.	0·328	0·331
Total nitrogen	0·438	0·381
Weight of heap, kilos	1270	1052
Period of storage	Jan. 7—April 14, 1915	
Loss	On initial quantity	Total N=100
Dry matter per cent.	87·4	15·6
Nitrogen as NH$_3$	+1·6	0·2
", other comps.	16·5	12·1
Total nitrogen	27·9	27·9
Temperature attained	40° C.	

Same manure, but stored for nine months

	Initial	Final
Moisture	77·5	80·5
Dry matter	22·5	19·5
Nitrogen as NH$_3$	0·079	0·007
", amide	0·031	0·039
", other comps.	0·328	0·428
Total nitrogen	0·438	0·474
Weight of heap, kilos	1270	587
Period of storage	Jan. 7—Oct. 11, 1915	
Loss	On initial quantity	Total N=100
Dry matter per cent.	95·9	17·2
Nitrogen as NH$_3$	41·0	2·9
", other comps.	39·8	30·1
Total nitrogen	50·2	50·2
Temperature attained	40° C.	

TABLE IX (*continued*).

	Cow manure Compact and under cover — Readily compacted		Cow manure Compact in the open — Readily compacted		Cow manure Compact and covered with soil in the open — Readily compacted		Cow manure Loose butt protected from both rain and wind — Moderately aerobic		Cow manure Loose protected from rain only — Moderately aerobic	
General character of heap										
Composition	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Moisture	81·1	79·8	81·5	79·1	81·5	79·4	81·5	75·7	82·0	71·0
Dry matter	18·9	20·2	18·5	20·9	18·5	20·6	19·5	24·3	18·0	29·0
Nitrogen as NH_3	0·101	0·008	0·094	0·010	0·094	0·011	0·079	0·048	0·091	0·021
,, amide	0·020	0·037	0·028	0·024	0·028	0·027	0·028	0·037	0·025	0·044
,, other comps.	0·285	0·336	0·288	0·348	0·288	0·353	0·308	0·372	0·299	0·523
Total nitrogen	0·415	0·371	0·410	0·382	0·410	0·391	0·415	0·457	0·405	0·588
Weight of heap, kilos.	2032	1632	2032	1612	2032	1455	2032	1241	2032	908
Period of storage	Nov. 29, 1915—	May 15, 1916	Nov. 29, 1915—	May 15, 1916	Nov. 29, 1915—	May 15, 1916	Nov. 29, 1915—	Jan. 22, 1917	Nov. 29, 1915—	Jan. 22, 1917
Loss	On initial quantity	Total N = 100								
Dry matter, per cent.	14·8		10·9		20·7		24·2		21·0	
Nitrogen as NH_3	93·6	22·8	91·6	21·0	91·6	21·0	62·7	12·0	87·1	17·3
,, amide	+1·6	+0·1	33·9	2·4	33·9	2·4	19·3	1·3	13·7	0·9
,, other comps.	7·9	5·4	3·8	2·7	11·8	8·3	26·2	19·4	14·3	10·5
Total nitrogen	28·1	28·1	26·1	26·1	31·7	31·7	32·7	32·7	28·7	28·7
Temperature attained	8° C.		—		—		24° C.			

	Mixed manure Loose under cover — Moderately aerobic		Mixed manure Loose in the open — Moderately aerobic		Mixed manure Trampled and thatched — Moderately anaerobic		Mixed manure Trampled in the open — Moderately anaerobic	
General character of heap								
Composition	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Moisture	76·8	72·6	76·5	72·0	72·4	70·3	72·4	70·4
Dry matter	23·2	27·4	23·5	28·0	27·6	29·7	27·6	29·6
Nitrogen as NH_3	0·063	0·020	0·090	0·015	0·149	0·119	0·149	0·086
,, amide	0·034	0·055	0·035	0·055	0·056	0·047	0·056	0·043
,, other comps.	0·301	0·512	0·405	0·434	0·565	0·653	0·565	0·630
Total nitrogen	0·398	0·587	0·440	0·504	0·770	0·819	0·770	0·759
Weight of heap, kilos.	2032	1265	1906	1108	5081	3037	5081	3150
Period of storage	Nov. 1, 1913—	May 20, 1914	Nov. 1, 1913—	May 20, 1914	May 27, 1915—	Oct. 19, 1915	May 27, 1915—	Oct. 19, 1915
Loss	On initial quantity	Total N = 100						
Dry matter, per cent.	26·5		30·6		35·6		33·4	
Nitrogen as NH_3	80·2	12·7	90·3	18·4	52·1	10·1	64·1	12·4
,, amide	+0·7	+0·1	8·5	7·2	50·8	3·6	52·4	3·8
,, other comps.	6·2	+4·4	22·3	7·8	30·8	22·7	90·8	22·7
Total nitrogen	8·2	8·2	33·4	33·4	36·4	36·4	38·9	38·9
Temperature attained							24° C.	

TABLE X. *Composition of farmyard manure.*

Dung from fatting or store, beasts (usually bullock dung).

	Ammoniacal nitrogen	Amide nitrogen	"Other" nitrogen	Total nitrogen	Dry matter	Ash	P_2O_5	K_2O	
Rothamsted	0·091	0·035	0·352	0·478	23·25	3·89	0·211	0·831	Average of 8 samples, 1915 heaps.
"	0·040	0·087	0·413	0·540	27·20	—	0·235	0·670	No cake, average 1904–13.
"	0·181	0·157	0·435	0·773	27·40	—	0·389	0·601	Cake fed.
Cambridge	0·028	—	0·290	0·318	22·85	—	0·075	0·855	Heifers, no cake. Wood, *Journ. Agric. Sc.* 1907–8, **2**, p. 207.
Cockle Park, Northumberland	0·203	—	0·371	0·574	24·10	—	0·190	—	Heifers, cake fed.
	0·150	0·090	0·510	0·750	27·25	—	0·270	0·68	*Five Years' Work,* Somerville. 1902. p. 68. (These beasts received 3 lbs. of cake and meal per head daily.)
Kilmarnock	0·070	0·058	0·535	0·663	28·23	5·57	0·374	0·823	Berry, *Bull.* 65. West of Scotland Agric. Coll.
Wooster, Ohio	—	0·404*	—	0·726	22·15	3·15	0·208	0·459	*Bull.* No. 246, 1912. Ohio Expt. Station.
Lauchstädt	0·186	0·070	0·521	0·777	28·00	·	0·412	0·820	Maercker and Schneidewind, *Landwirt. Jahrbuch* 27, p. 215.
"	0·164	0·052	0·392	0·608	23·02	—	0·263	0·716	
Average	0·124	0·078	0·425	0·621	25·35	—	—	—	
Dung from milking beasts (cow dung).									
Rothamsted	0·071	0·031	0·260	0·362	18·71	3·70	0·180	0·562	Mean of 10 samples, 1914 heaps.
"	0·088	0·028	0·306	0·422	18·74	3·81	—	—	Mean of 8 samples, 1915–16 heaps.
Kilmarnock	0·076	0·012	0·333	0·421	19·96	5·52	0·229	0·421	Berry. *Bull.* 65. West of Scotland Agric. Coll.
"	0·074	0·026	0·254	0·354	18·30	5·27	0·192	0·296	
West of Scotland	0·064	—	0·282	0·346	19·95	4·27	0·266	0·381	Average of 12 samples from dairy farms. See above.
Wooster, Ohio	0·285*	—	—	0·572	20·95	2·45	0·100	0·520	*Bull.* No. 246, 1912. Ohio Expt. Station.
Lauchstädt	0·173	0·040	0·302	0·515	—	—	—	—	Maercker and Schneidewind, *Landwirt. Jahrbuch* 27, p 21.
Average	0·091	0·027	0·290	0·427	19·44	4·17	0·193	0·436	
Horse dung									
Rothamsted	0·096	0·039	0·307	0·442	25·13	4·57	0·243	0·727	Mean of 4 samples, 1914 heaps.
London (stable manure)	0·080	—	0·460	0·540	23·91	4·6	0·330	0·450	B. Dyer, *Journ. Agric. Sc* Vol. I, p. 108.
Army manure heaps 1916	0·119	—	—	0·508	[50·03]†	[27·33]†	0·310	0·790	Russell, *Journ. Bd. Agric.* 1917, **23**, 1053.
Kilmarnock	0·045	0·022	0·275	0·342	22·75	6·01	0·203	0·271	Straw litter. } Berry, *loc. cit.*
"	0·082	0·020	0·326	0·428	21·73	4·41	0·193	0·337	Moss litter. }
Wooster, Ohio	—	0·365*	—	0·695	40·84	5·30	0·108	0·636	*Bull.* No. 246, Ohio Expt. Station.
Lauchstädt	0·384	0·062	0·350	0·796	—	—	—	—	
Average	0 134	0·036	0·344	0·536	26·87	4·98	0·231	0·535	

† High because of sand and included in manure. These figures are omitted from the average.

General Summary.

	Total nitrogen	Ammoniacal nitrogen	Amide nitrogen	More complex nitrogen compounds	Phosphoric acid (P_2O_5)	Potash (K_2O)
Bullock	0·62	0·12	0·08	0·42	0·26	0·72
Cow	0·43	0·09	0·03	0·29	0·19	0·44
Horse	0·54	0·13	0·04	0·34	0·23	0·54

* Water soluble.